Centro de Ciências do Mar do Algarve
Algarve Centre of Marine Sciences
Annual Report
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Publications List

Division Aquaculture and Biotecnology

Articles in international refereed journals (listed in SCI)

Abbink, W., Bevelander, G. S., Rotllant, J., Canario, A. V. M. and Flik, G. (2004) Calcium handling in Sparus auratus: effects of water and dietary calcium levels on mineral composition, cortisol and PTHrP levels. *Journal of Experimental Biology* **207**, 4077-4084.

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Aragão, C., Conceição, L. E. C., Martins, D., Ronnestad, I., Gomes, E. and Dinis, M. T. (2004) A balanced dietary amino acid profile improves amino acid retention in post-larval Senegalese sole (Solea senegalensis). *Aquaculture* **233**, 293-304.

Aragão, C., Conceição, L.Conceição, L. E. C., Fyhn, H. J. and Dinis, M. T. (2004) Estimated amino acid requirements during early ontogeny in fish with different life styles: gilthead seabream (Sparus aurata) and Senegalese sole (Solea senegalensis). *Aquaculture* **242**, 589-605.

Campinho, M. A., Moutou, K. A. and Power, D. M. (2004) Temperature sensitivity of skeletal ontogeny in Oreochromis mossambicus. *Journal of Fish Biology* **65**, 1003-1025.

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Guerreiro, P. M., Fuentes, J., Flik, G., Rotllant, J., Power, D. M. and Canario, A. V. M. (2004) Water calcium concentration modifies whole-body calcium uptake in sea bream larvae during short-term adaptation to altered salinities. *Journal of Experimental Biology* **207**, 645-653.

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Leite, R. B., Afonso, R. and Cancela, M. L. (2004) Perkinsus sp infestation in carpet-shell clams, Ruditapes decussatus (L), along the Portuguese coast. Results from a 2-year survey. *Aquaculture* **240**, 39-53.

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Morais, S., Conceicao, L. E. C., Dinis, M. T. and Ronnestad, I. (2004) A method for radiolabeling Artemia with applications in studies of food intake, digestibility, protein and amino acid metabolism in larval fish. *Aquaculture* **231**, 469-487.

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Riviere, G., Michaud, A., Deloffre, L., Vandenbulcke, F., Levoye, A., Breton, C., Corvol, P., Salzet, M. and Vieau, D. (2004) Characterization of the first non-insect invertebrate functional angiotensin-converting enzyme (ACE): leech TtACE resembles the N-domain of mammalian ACE. *Biochemical Journal* **382**, 565-573.

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Simes, D. C., Williamson, M. K., Schaff, B. J., Gavaia, P. J., Ingleton, P. M., Price, P. A. and Cancela, M. L. (2004) Characterization of osteocalcin (BGP) and Matrix Gla Protein (MGP) fish specific antibodies: Validation for immunodetection studies in lower vertebrates. *Calcified Tissue International* **74**, 170-180.

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Articles in other refereed journals (national and international)

Laizé V. et al. (2004) Magnet Assistant Lipofection of fish bone derived VSa13 cells.IBA BioTAGnology

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1. Power, D.M. Canario, A.V.M., Amaral J., Balment, R., Flik, G. And Clark, M. Calcium the backbone of fish culture: importance in skeletal formation, reproduction and normal physiology. Aquaculture Europe: Biotechnology for Quality. Barcelona, Espanha, October 2004. European Aquaculture Society. Special publication No.34, 2004 (oral DMP).
2. Adelino V M Canário**,** Eleftherios Zouros and Filip A M J Volckaert. European Network of Excellence "Marine Genomics Europe": a contribution to Aquaculture. Aquaculture Europe: Biotechnology for Quality. Barcelona, Espanha, October 2004. European Aquaculture Society. Special publication No.34, 2004 (oral)

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Santos R, Cunha A, Cabaço S and Alexandre A. Influência das actividades antropogénicas nos campos de ervas marinhas na Ria Formosa. II Reunião Científica da Rede CYTED-XVII Sobre Zonas Húmidas, 3º Simpósio Interdisciplinar Sobre Processos Estuarinos (SIPRES), Algarve, Portugal.
Santos R. Commercial seaweeds for water treatment of marine animal holding units. SEMAPP - Science, Education and Marine Archaeology Program in Portugal, Ocean Technology Foundation, Zoomarine, Portugal.
Schuenhoff, Mata & Santos "An unexploited method of water treatment for marine aquaria: Fluidized seaweed biofiltration" at Aquality 2004, Lisbon Oceanario, 1. - 6. April 2004
Schuenhoff A, Mata L, and Santos R. Double duty: read seaweed with strong natural antibiotic properties yields a valuable product and functions as biofilter for mariculture effluent. Aquaculture Europe 2004 Conference on "Biotechnologies for quality", Barcelona, Spain.

Project and consultancy final reports

Barracosa, H. (2004) Fundamentação e Avaliação da Educação Ambiental não formal: estudo de caso da ecoteca de Olhão, Relatório Final de projecto, Protocolo Instituto do Ambiente e CCMAR.
Silva J, Cunha A, Santos R (2004). Caracterização dos bancos de fanerogâmicas e macroalgas no estuário do Rio Arade. Contrato Instituto Portuário do Sul/CCMAR, 14pp.
Santos R, Mata L and Schuenhoff A (2004). Species diversification and improvement of aquatic production in seaweeds purifying effluents from integrated fish farms, SEAPURA, EU Contract Q5RS-2000-31334, final report of the Portuguese partner, 34pp.

Prizes and Honours

List of thesis supervised by members of the research unit

Division of Aquaculture and Biotechnology

Theses PhD

Completed

Aragão Teixeira, Cláudia (2004). Towards the assessment of amino acid requirements in seabream and sole larvae. Universidade do Algarve (supervisors Maria Teresa Dinis and Luis E.C. Conceição).

Brinca, L. (2004). Regulação por neuropeptideos da secreção e síntese da hormona de crescimento e da prolactina em pituitárias de dourada (*Sparus aurata* L.). Universidade do Algarve (Supervisor: Deborah Power).

Cardoso, J.C.R. (2004). Estudo da estrutura e função genética dos receptores acoplados a proteínas-G no peixe balão. Universidade do Cambridge, UK (Supervisors: Deborah Power and Melody Clark).

Ongoing

Ascenso, Rita Margarida Teixeira: "Identification of *P. Atlanticus* genes differentially expressed in response to parasite-host interaction and development of an in vivo infestation system". (Supervisor: Leonor Cancela). Completion expected in 2007.

Borges, Gisela. Endothelium dysfunction in microvascular diseases. (Supervisor: Josefina Coucelo). Completation expected in 2006.

Carvalho, Suzana. Papel das Comunidades de Macrofauna Bentónica na Gestão de Tanques para Piscicultura (supervisors Maria Teresa Dinis and Luis Fonseca). Completion expected in 2005.

Coesel, Sacha. Isolation and characterization of regulatory and biosynthetic genes involved in carotenogenesis in the microalga *Dunaliella salina.* (supervisors João Varela and Chris Bowler). Completion expected in 2006.

Fagundes, Teresa Ecologia comportamental do blenídeo *Salaria pavo* na Ria Formosa: tácticas alternativas de reprodução e inversão de papéis sexuais (Supervisors Rui Oliveira and Adelino Canário). Completion expected in 2005

Frade, Pedro Alexandre. Chemical identification and function of pheromones in the reproduction of tilapia, *Oreochromis mossambicus* (Pisces: Cichlidae). Universidade do Algarve (supervisors Eduardo N. Barata, Adelino V.M. Canário and Peter C. Hubbard). (Fellowship temporarily suspended).

Gavaia, Paulo J. "Functional analysis of osteocalcin (Bone Gla protein, BGP) from bony fish during skeletal development. Universidade do Algarve (Supervisors: Leonor Cancela and Carmen Sarasquete, CSIC Cadiz, Espanha). Completion expected in 2005.

Henriques, Nuno. Regulation of the gene expression associated with the carotenoid biosynthesis in the microalga *Dunaliella salina*. Universidade do Algarve. (Supervisors: Leonor Cancela and João Varela). Completion expected in 2005.

Mira, Sara Maria. Population genetics of an endangered species, the Bonelli's eagle (*Hieraaetus fasciatus*). (Supervisors: Leonor Cancela and Pedro Beja in Portugal; Paula Dias/CNRS – Montpellier, França). To be completed in 2006.

Morais, Sofia. Digestive and assimilation capacity of marine fish larvae with respect to dietary lipid/protein ratios and lipid quality. (Supervisors Maria Teresa Dinis, Luis E.C. Conceição and Ivar Rønnestad). Completion expected in 2005.

Pais, Miguel Caldeira. Seleção e utilização do habitat pela popºulação não reprodutora de Aguia de Bonelli (Hieraaetus fasciatus) no Sul de Portugal. (Supervisors: M. Leonor Cancela and Pedro Beja (Erena]) Completion in 2006.

Pinto, Patricia Isabel Silvestre - Diversidade, expressão e mecanismo de acção do receptor de estrogénio na dourada, Sparus aurata. Universidade do Algarve (supervisor Adelino V.M. Canário). Completion expected in 2005.

Ramos, Alexandra. Isolation and characterization of the lycopene cyclase gene involved in the carotenoid biosynthetic pathway in the microalga *Dunaliella salina.* (supervisors João Varela and Bertram Brenig). Completion expected in 2007.

Serafim, Maria Paula. Universidade do Algarve (supervisor Margarida Castro). Completion expected in 2006.

Serrano, Rui Manuel. Pheromones in the reproduction of *Salaria pavo* and *S. fluviatilis* (Pisces: Blenniidae): a comparative study. Universidade do Évora (supervisors Eduardo N. Barata, Adelino V.M. Canário and Peter C. Hubbard). Completion expected in 2005.

Soares, Sandra Sofia Ganchas. Stresse oxidativo no músculo cardíaco induzido por iões metálicos. (supervisors: Josefina Coucelo, Manuel Aureliano Alves e Carlos Gutierrez-Merino). Completation expected in 2006.

Tiago, Daniel António Martins: Role of IGF1 and IGF2 in the mineralization mechanisms using fish bone derived cell lines. (Supervisors M.L. Cancela and V. Laizé from CCMAR, MA Alves from FCT-UALG) To be completed in 2007.

Vasconcelos, Paulo. Universidade do Algarve (supervisor Miguel Gaspar, IPIMAR and Margarida Castro. Completion expected in 2005.

Theses Master of Science

Completed

Anastassiades, George. Early weaning of sole, *Solea senegalensis*, onto microencapsulated diets, with different levels of protein hydrolysates. MSc student at the University of Ghent (Belgium). (Supervisors: Maria Teresa Dinis, Luís Conceição and Laura Ribeiro).

Fidalgo, João Pedro Marçal. (2004) "Identificação e clonagem de um intrão adicional no gene da proteína Gla da matriz de peixe zebra: Implicações na regulação genética". Masters in Aquaculture, University of Cadiz, Spain." (Supervisor: ML Cancela)

Inácio, Ana Rita da Silva (2004). "Selecção de áreas prioritáriaspara a conservação da biodiversidade no sul de Portugal". Masters in Gestão e Conservação da Natureza, Universidade do Algarve. (Supervisors: Pedro Beja, ERENA,M Leonor Cancela, CCMAR/UALG)

Leite, Ricardo Mário Bastos. (2004) "Clonagem e regulação de um gene envolvido no metabolism das purine de Perkinsus olseni / atlanticus. Masters in Aquaculture, University of Cadiz, Spain. (Supervisor: ML Cancela)

Patrícia, Joana Dias. (2004) "Effect of infection by the parasite Perkinsus atlanticus (Perkinsea) in the processus of gameta release and in the biochemical quality and viability of resulting eggs and larvae Masters in Aquaculture, University of Cadiz, Spain.". (Supervisor: ML Cancela)

Ongoing

Cepo, Susana Isabel Coelho dos Santos. "Contributo para a implementação de um sistema de gestão integrado de recolha e valorização de óleos alimentares usados". Masters in Gestão e Conservação da Natureza, Universidade do Algarve. Supervisors: Raul Costa, António Portugal and M Leonor Cancela). Completion in 2005.

Fonseca Vera. Identification of genes differentially expressed during the mineralization of fish bone-derived cell lines. Mestrado em Biotecnologia, Universidade do Algarve (Supervisors: M.L.eonor Cancela and Vincent Laizé). Completion expected in 2005

Pombinho, António Ribeiro. Effect of extracellular calcium on MGP gene expression. Mestrado em Biotecnologia, Universidade do Algarve. (Supervisors: Leonor Cancela and Vincent Laize). Completion expected in 2005.

Santos, Erika Silva. "Potencial de utilização do Cistus ladanifer L. na vegetalização de áreas mineiras" . Masters in Gestão e Conservação da Natureza, Universidade do Algarve. Supervisors. Mª Manuela Abreu, Cristina Nabais and ML Cancela). Completion in 2005.

Silva, Conceição. Desenvolvimento de ferramentas interactivas para divulgação das aplicações da biotecnologia ambiental em biorremediação. MSc Biotechnology, Universidade do Algarve (co-orientação. (Supervisors: C. Rocha and M. L. Cancela CCMAR/UALG). Completion expected in 2005.

Graduation Honours thesis (Estágio de licenciatura)

Completed

Cavaco, Sofia Isabel (2004) Efeito de extractos de nacre da ostra Crassostreia gigas na mineralização de células de osso de Sparus aurata. (Supervisors: M Leonor Cancela e Dina Simes)

Correia, Sónia (2003/2004). Estudo da ontogénese do esqueleto e do sistema digestivo em larvas de pargo (*Pagrus pagrus*) em condições de cultivo. (Supervisors: Maria Teresa Dinis, Luís Conceição and Florbela Soares).

Costa, Ricardo (2004). Estudo do efeito das microalgas na ingestão de alimento em larvas de peixes marinhos. (Supervisors: Maria Teresa Dinis and Laura Ribeiro)

Couto, Ana (2003/2004). O efeito das microalgas na actividade dos enzimas digestivos de larvas de peixes marinhos. (Supervisors: Maria Teresa Dinis and Laura Ribeiro).

Dias, Maria de Lurdes Duarte (2004). Influência do plano alimentar na adaptação a alimento inerte e crescimento do linguado (*Solea senegalensis,* Kaup 1858). (Supervisors: Maria Teresa Dinis, Luís Conceição and Sofia Engrola).

Domingues, Susana Filipa (2004). Estudo do efeito do citral na morfologia do esqueleto de peixe zebra durante os primeiros estádios de desenvolvimento. (Supervisors: M. Leonor Cancela, Paulo Gavaia).

Figueiredo, Joana Guimarães (2004). Controlo hormonal do sinal fermonal em machos de tilapia Oreochromis mossambicus. (supervisors: Peter Hubbard Adelino V M Canario).

Figueiredo, Joana Isabel (2004) Estudo do metabolismo do cálcio e do efeito da PTHrP no transporte de cálcio no intestino da dourada Sparus auratus. (supervisors: Juan Fuentes e Adelino V M Canario).

Leal, Leonor Jorge Silva (2004). Cultivo larvar de *Solea senegalensis*: parâmetros zootécnicos. (Supervisors: Maria Teresa Dinis and Florbela Soares).

Massarico, Sandra Paula Pereira Gonçalves (2004). Ectoparasitas em cultivos de dourada (*Sparus aurata*) e robalo (*Dicentrarchus labrax*). (Supervisors: Florbela Soares).

Mestrinho, Inês Manuel Fernandes (2004). Início do estudo fitoquímico de *Cecropia purpurascens (Urticaceae)* Berg: Rastreio e identificação de compostos com actividade antioxidante. (Supervisor: João Varela).

Pardelha, Bruno Miguel Guerreiro (2004) Sequenciação e modelação de duas sequências da BGP de peixes. (Supervisors: M. Leonor Cancela, Vincent Laizé and Paulo Martel [FCT-UALG]).

Pinto, Elsa (2004). Clonagem e expressão de enzimas do citocromo P450 de peixe. (supervisor: Adelino V M Canario).

Rafael, Marta Isabel da Silva (2004) Clonagem do cDNA da proteína morfogenética do osso-2 (BMP-2) de Sparus aurata: Estudo da sua expressão genética e análise da conservação de domínios proteicos. (Supervisors: M. Leonor Cancela and Vincent Laizé)

Simões, Brigite (2004) Clonagem e estrutura do gene do colagénio X α1 do peixe zebra, *Danio rerio*. Análise funcional do promotor. (Supervisors: M. Leonor Cancela and Natércia Conceição)

Viana, Luís Manuel Fernandes (2004). Efeito de Imunoestimulantes e Tratamentos Profilácticos na Produção de Pós-Larvas e Juvenis de Linguado (*Solea senegalensis*, Kaup 1858). (Supervisors: Maria Teresa Dinis, Luís Conceição and Sofia Engrola).

Ongoing

Baptista, Bruno (2005). Efeito da utilização de microalgas em diferentes fases do desenvolvimento de larvas de peixes marinhos (Supervisors: Maria Teresa Dinis and Laura Ribeiro). Completion expected in 2005.

Brito, Anabela Ben'Simon. Clonagem e estudos de regulação da expressão do gene da phd1/egln-2 (prolyl hydroxylase domain protein 2) do parasita Perkinsus atlanticus (Supervisors: M. Leonor Cancela and Ricardo Leite)

Carneiro, João Tiago Estêvão Tomé. Contribuição para a optimização de uma metodologia de detecção de cistos de Cryptosporidium spp em amostras de águas (Supervisors: M. Leonor Cancela and Ricardo Leite)

Coelho, Nélson Alexandre Castilho. Transdiferenciação de osteoblastos de Sparus aurata em adipócitos (Supervisors: M. Leonor Cancela and Vincent Laizé). Completion in 2005.

Costa, Isabel. Metabolic Capacity and Stress Response of five North Atlantic Teleost species. (Supervisores: Natércia Joaquim, Josefina Coucelo (UAlg) and Kurt Gamperl (Ocean Sciences Centre, MUN, Canada)

Dias, Paulo José Sousa. Clonagem e comparação da expressão da osteocalcina de tamboril e tainha com as de outros peixes osseos. (Supervisors: M. Leonor Cancela e Carla Viegas)

Martins, Vânia F. Estudo comparativo de metazoas de metazoários: hormonas da tiróide. (Supervisor: Deborah Power)

Pereira, Susana Cristina Oliveira. Identificação de polimorfismos do gene da MGP humana em populações de risco para desenvolvimento de problemas cardiovasculares. (Supervisors: M. Leonor Cancela e Jorge Rocha, IPATIMUP)

Santos, Jose Beirão (2005). Microinjeção de crioprotectotes em embriões de dourada, *Sparus aurata* (Supervisors: Maria Teresa Dinis and Elsa Cabrita). Completion expected in 2005.

Simão, Márcio Alexandre. Estudo do efeito da dinâmica bêntica de Ferro na resposta da ameijôa Ruditapes decussatus a uma infecção pelo parasita Perkinsus olseni (Supervisors: Carlos Rocha and M. Leonor Cancela)

Soares, Rita I. Identificação e caracterização das proteínas da matriz extracelular: uma baordagem molecular e proteómica. (Supervisor: Deborah Power)

Division of Living Resources

Theses PhD

Completed

Dias, Ana Natália. Ecology of the saltmarsh fauna in the Ria Formosa lagoon. Universidade do Algarve (supervisor Martin Sprung).

Silva, J (2004) The photosynthetic ecology of *Zostera noltii.* PhD thesis. Faculdade de Ciências do Mar e do Ambiente, Universidade do Algarve, 87 p (supervisor Rui Santos).

Ongoing

Alberto, Filipe. Population genetics, clonal structure and phylogeography of the seagrass *Cymodocea nodosa* using microsatellite markers. Univ. Algarve (supervisor E. Serrão, Univ. Algarve, and C. Duarte, CSIC, Spain). Completion expected in 2004.

Amaral, A. "Ecofisiologia de *Ruditapes decussatus* na Ria Formosa" (Supervisor Luís Chícharo Co-orientação com o Doutor Uxio Labarta (CSIC – Vigo) (Espanha).

Aníbal, Jaime. Effects of the seasonal dynamics of green algae of the nutrient flux in the sediments of the Ria Formosa lagoon. Universidade do Algarve (supervisor Martin Sprung). Completion expected in 2004

Beldade, M.R.do Ó. De O. Padrões de recrutamento e estabilidade em comunidades de peixes crípticos das costas rochasas. (supervisors: Emanuel Gonçalves (ISPA) and Karim Erzini). Completion expected in 2006.

Berecibar, Estibaliz. "Global related changes in the Portuguese marine flora". Universidade do Algarve (Supervisor: Rui Santos, CCMar).

Billard, Emmanuelle. Evolution of reproductive strategies in four closely related brown seaweeds, *Fucus spiralis, F. vesiculosus, F. ceranoides* and *F. serratus*. Univ. Algarve and Univ. Paris VI, France. (Supervisors: Ester Serrão and Myriam Valero, CNRS, France). Completion expected in 2007.

Borges, R. "Processo de retenção do ictioplancton na costa da Arrábida (supervisors: Emanuel Gonçalves-ISPA, Alexandra Chícharo Universidade do Algarve) - Bolseiro da FCT (started in 2003).

Cabaço, S. "Population dynamics of *Zostera noltii* along a nutrient gradient". Universidade do Algarve (supervisors: Rui Santos, CCMar and Carlos Duarte, Universidade das Ilhas Baleares).

Campos, Aida. (2003). The estimation and improvement of the selectivity in crustacean and fish trawls. (Supervisor: Karim Erzini). Completed in January 2004.

Candeias, A. "The Processes Of Feeding In The Physiological Energetics Of Coastal Meroplankton" (Supervisor Alexandra Chícharo em co-orientação com Doutor Andrew Bruce Yule - School Of Ocean Sciences, University of Wales).

Coelho, R. Biologia, dinâmica espacio-temporal, gestão e conservação de tubarões de profundidade. (Supervisor: Karim Erzini). Completion expected in 2006.

Costa, M.E. By-catch e rejeições da pesca comercial de arrasto na costa Sul de Portugal. Universidade do Algarve (supervisor Teresa Cerveira Borges)

Díaz-Almela, Elena. "Population dynamics and reproductive ecology of *Posidonia oceanica* (Delile)". Univ. Illas Ballears, Spain (Supervisors: Ester Serrão and Carlos Duarte, CSIC, Espanha). Completion expected in 2005.

Esteves, Eduardo Bruno Oliveira. Recrutamento e condição larvar de savelha, *Alosa fallax fallax*, nos rios Mira e Guadiana. (Supervisor: J. Pedro Andrade).

Fonseca, P. J. M. R. da. Selectividade de redes de arrasto e emalhar na costa continental Portuguesa. (Supervisor: Karim Erzini). Completion expected in 2006.

Godinho, C. "The impact of anti-fouling paints on seagrass populations of *Zostera noltii* (Hornem., 1832) in the Ria Formosa Lagoon". Universidade do Algarve (supervisors: Rui Santos, CCMar and Maria João Bebianno).

Hazin, H. Influência das variáveis oceanográficas na dinâmica populacional do espadarte, *Xiphias gladius*, no Oceano Atlânico. (Supervisor: Karim Erzini). Completion expected in 2005.

Lago-Leston, Asuncion. The Molecular Basis for Differential Stress-Tolerance in Co-Existing, Ecologically Similar Algal Species, Univ. Algarve (supervisor G. Pearson, and E. Serrão). Completion expected in 2006.

Leitão, F.M. de S.- "Contribuição dos recifes artificiais da costa Algarvia na ecologia trófica de sparídeos." (Supervisors: Miguel Neves dos Santos (IPIMAR) and Karim Erzini). Completion expected in 2004.

Lino, P.G. Potencial do repovoamento piscícola na costa Sul do Algarve com espécies marinhas autóctones produzidas em cativeiro. Supervisor: K. Erzini, start: 2004.

Mata, Leonardo - "Estudo da fisiologia de *Falkenbergia rufolanosa* para a optimização da produção e valorização da biomassa cultivada com os efluentes de uma piscicultura." Universidade do Algarve (Supervisor: Rui Santos).

Machás, Raquel. "The role of *Zostera noltii* on the food web of Ria Formosa", Universidade do Algarve (supervisor: Rui Santos).

Marçalo, A. Avaliação de stress em sardinha (*Sardinha pilchardus*) durante a pesca do cerco. Supervisor: K. Erzini, Co-supervisor: Dr. Yorgos Stratoudakis, IPIMAR, start: 2004.

Marques, Alexandra. Population dynamics of dominant copepod species in the Ria Formosa lagoon. University of Bangor (supervisors Andy Yule and Martin Sprung). Completion expected in 2004

Mendes, J.C "Long-Term Time Series of Continuous Plankton Recorder Survey off Portuguese Coast" (Supervisor Alexandra Chícharo em co-Orientação com o Doutor Miguel Santos do IPIMAR e com o Doutor Chris Reid, do SAPHOS (Plymouth).

Morais, P. - "*Engraulis encrasicolus* (Linnaeus, 1758) population dynamics in the Guadiana estuary and adjacent coastal area" (Supervisores Alexandra Chícharo e Luis Chícharo).

Moschino, V. - "Impact of fishing activity on the morphology, physiology and biochemistry of the bivalves *Chamelea gallina* and *Tapes philippinarum* from coastal and lagoon areas of the Northern Adriatic Sea (Italy)" (Supervisor Luis Chícharo em co-orientação com a Dra. Maria Gabriella Marin da Universidade de Padova (Itália). Não tem data

Pais, MC. Use and selection of habitats by non-breeding Bonelli's eagles in southern Portugal. (supervisors Pedro Beja and Leonor Cancela). Completion expected in 2006.

Ribeiro, J. Ecologia e dinâmica da ictiofauna da Ria Formosa. Universidade do Algarve (supervisor: Karim Erzini). Completion expected in 2006.

Santana, J. - "Comparação bioeconómica das pescas no rio Tocains Amazónia-Brasil", (Supervisor Luis Chícharo em co-orientação com o Doutor Miguel Petrere da Universidade de Pernambuco (Brasil). Não tem data

Schuenhoff, Andreas - "The application of *Asparagopsis-Falkenbergia* as a commercially viable biofilter for water re-use". Universidade do Algarve (Supervisors: Rui Santos, CCMar and James Muir, Universidade de Stirling).

Serafim, Maria Paula. Universidade do Algarve (supervisor Margarida Castro). Completion expected in 2006.

Stobberup, K. Study of community structure, trophic interactions and exploitation pattern in the Cape Verde coastal ecosystem. (Supervisor: Karim Erzini). Completion expected in July 2005.

Teodósio, J. "Dinâmica populacional e caracterização do estado fisiológico e bioquímico da ameijoa asiática Corbicula fluminea na bacia hidrográfica do rio Guadiana". (Supervisores Alexandra Chícharo e Luis Chícharo). Não tem data

Vasconcelos, Paulo da Conceição Silva (supervisors Miguel Gaspar (IPIMAR) and Margarida Castro). Completion expected in 2006.

Theses Master of Science

Completed

Alexandre, Ana. Impacto da actividade de marisqueio na floração e no esforço de reprodução sexual em Zostera noltii Hornemann na Ria Formosa Mestrado em Estudos Marinhos e Costeiros (Ramo de Recursos Biológicos), Universidade do Algarve (supervisores Rui Santos e Ester Serrão).

Alexandre, Ana. Impacto do marisqueio na reprodução da fanerogâmica marinha *Zostera noltii*. Univ. Algarve (supervisor: R. Santos and Ester Serrão). 2004.

Corado, M. (2004). Estudo da ictiofauna na interface estuário-mar através de censos visuais subaquáticos. Master Internacional de Pesca y Acuicultura da Universidad de Cádiz. Supervisor: Jorge M.S. Gonçalves (CCMAR/FCMA, Universidade do Algarve).

Malaquias, A.A.E. Contribuição para o conhecimento da sistemática e da ecologia do género Haminoea Turton and Kingston, 1830 (Mollusca: Gastropoda: Cephalasidea). Mestrado em Ecologia, Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

Olim, S. (2003) Biologia e morfometria de várias espécies da família Triglidae na costa Sul de Portugal. (Supervisor: Teresa Cerveira Borges).

Vieira, Vasco. Modelos populacionais da alga *Gelidium sesquipedale*., Mestrado de Estudos Marinhos e Costeiros, Universidade do Algarve (supervisor Rui Santos).

Ongoing

Bentes, L. Essential fish habitats. Supervisor: Karim Erzini. CCMAR/FCMA, Universidade do Algarve. Completion expected in 2005.

Ferreira, R. Influência de parâmetros oceanográficos e pesqueiros na captura acidental de tartarugas marinhas pela pesca de espadarte nos Açores. Completion expected in 2004 Supervisor: Karim Erzini.

Frutuoso, Ana Luísa. Diversidade de Fungos Ectomicorrízicos: relação com Gestão Florestal, Univ. Algarve (supervisors: M. Honrubia, P. Beja, E. Serrão). Completion expected in 2005.

Rosa, Ana. Guia de Campo das algas do intertidal da Praia da Vigia., Mestrado de Biologia e Geologia para o Ensino, Universidade do Algarve (supervisor Rui Santos).

Saldanha, H. Ghost fishing of hake gill nets in the Algarve. (Supervisors: Karim Erzini and Miguel Neves dos Santos (IPIMAR)). Completion expected in 2004.

Santos, V. A socio-economic study of a fishing community in India. (Supervisors: Karim Erzini and Maarten Bavink). Completion expected in 2004.

Graduation Honours thesis (Estágio de licenciatura)

Completed

Almeida, Cheila (2004). Caracterization of the macro epifauna associated with sandy substrates off the Central Algarve. Estágio de Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve. Supervison: Karim Erzini and Jorge Gonçalves.

Ana Maria Leocádio (2004). Estrutura do stock da lagosta Panulirus argus (Latrille, 1804), na região do Grande Caribe. Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve. (Supervisors: Margarida Castro + Raúl Cruz (U. de Havana, Cuba)).

Araújo, João (2004). Survival of fishes that escaped through by-catch reduction devices in the purse seine fishery. Estágio de Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve. Supervison: Karim Erzini and Jorge Gonçalves.

Armindo Neves Lourenço (2004). A Inspecção, vigilância e controlo da pesca nos portos do Continente. Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve. (Supervisors: Margarida Castro + Pedro Ferreira (Direc. Regional das Pescas, Olhão)).

Barata, M.Araújo, João (2004). Filogeografia do cágado *Mauremys lepro*sa no território português.Survival of fishes that escaped through by-catch reduction devices in the purse seine fishery. Estágio de Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve, Faro. (Supervisor: Rita Castilho). Supervison: Karim Erzini and Jorge Gonçalves.

Barradas, António (2004). Estudos de Reprodução do Carapau do Cunene (*Trachurus trecae*) nas águas de Angola. Universidade Agostinho Neto, Angola (Supervisors: Filomena Vaz-Velho from the Instituto de Investigação Marinha of Angola; Isabel Afonso-Dias, CCMar; Vitório Samuel from the Faculdade de Ciências da Universidade Agostinho Neto).

Botelho, P.F. (2004) Contribution to the study of ichthyofauna of the Ria Formosa lagoon system. Tese de Licenciatura. Faculdade de Ciências do Mar e do Ambiente da Universidade do Algarve, Faro. (Supervisores: Per J Palsbøll, University of Berkeley, USA.and Rita Castilho). Supervison: Karim Erzini and Jorge Gonçalves.

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Description of the Research activities

Division of Aquaculture and Biotechnology

Group: Molecular Biology of Marine Organisms

Leader: Leonor Cancela
Visiting Scientist: Ivar Ronnestad
Researchers and post docs: Vincent Laizé, Laurence M. Elandalloussi, Juan B. Ortiz Delgado, Pedro M. Rodrigues, Natércia Conceição, Dina Simes, Sandra P. Marques, Vanesa Robles

PhD students: Paulo Gavaia, Nuno Henriques, Sara Mira Silva, Daniel Tiago, Rita Ascenso
MSc students: António Pombinho, Vera Fonseca
Technicians/ research assistants: Carla Viegas, Ricardo Leite, Ricardo Afonso, João Fidalgo, Daniel Braga
Undergraduate students: Brian Schaff, Susana Domingues, Bruno Pardelha, Brigite Simões, Sofia Cavaco, Marta Rafael, Anabela Brito, Nelson Coelho, Susana Pereira, João Carneiro, Márcio Simão.

Summary of activities and progress during 2004

The major theme of our group research relates to study of regulatory pathways envolved in bone and cartilage cellular differentiation and molecular adaptations to physiological and environmental stress. Various aquatic organisms are used as models systems with an emphasis on fish and amphibians. Specific genes currently being used as molecular markers for bone and cartilage have been cloned from all model organisms (non-mammalian) presently in study in our laboratory and their sites of gene expression and protein accumulation identified. Functional analysis of promoter regions through construction of specific deletion mutants, site-directed mutagenesis and electrophoretic mobility shift assays has led to the identification of previously undetected DNA regulatory regions in selected genes of interest. These results will further permit the identification of the nuclear factors involved in specific gene regulation. Following the recent development of fish bone- and cartilage-derived cell lines in our laboratory, major emphasis is presently directed towards understanding mechanisms involved in bone and cartilage cell differentiation, role of Gla protein in extracellular matrix mineralization and response to environmental parameters through the use of integrated genomic/proteomic approaches.

A second project was initiated more recently on host–parasite interaction using as model organism the parasite *Perkinsus atlanticus* and its natural host the clam *Ruditapes decussatus*. A clonal cell culture of *Perkinsus atlanticus* was developed and good progress was made towards initiating studies on the biology of the parasite and host-parasite interactions using an integrated approach envolving histological, molecular and cellular biology techniques. Molecular diagnostic tools were developed to detect parasite infection in host tissues and environmental factors affecting parasite growth and development. A three year epidemiological study on key sites of the Portuguse coast concerning culture of bivalves was performed to monitor the extent of the infection in the Portuguese coast and is currently being continued but limited to the southern coastal region.

Molecular markers for studies on genetic variability, paternity and sex determination were also developed in particular for studies on local endangered species. Two projects are currently on going, concerning 1) the development of molecular markers for studies on molecular sexing, identification and genetic variability of the Bonelli's eagle and 2) the use of molecular markers for measuring genetic variability and estimating the effective population size of the coastal otter in South-western Portugal.

Plan for 2005

1. Molecular determinants of extracellular matrix calcification

Main purposes: Research focus is primarily on i) understanding molecular pathways of tissue mineralization and its regulation in adult life and during development, using as model organisms fish (gilthead seabream and zebrafish) and amphibian (xenopus), and ii) the role of vitamin K dependent proteins (matrix Gla and bone Gla proteins) in this process. Additional goals include iii) studies on the effect of genetic or environmental factors on alterations of the normal pattern of tissue calcification and its relation with altered expression of vitamin K-dependent proteins and iv) molecular adaptations of mechanisms that control extracellular matrix mineralization throughout evolution. In order to perform studies on bone- and cartilage-related protein function, gene expression/regulation and functional promoter analysis, it was imperative to obtain bone- and

cartilage-derived cell lines from fish and amphibian, not available at present. For this purpose fish and amphibian derived cell lines were developed and transfection conditions for these cell lines as well as primary cells cultures were optimized in order to be able to analyse specific gene expression in vitro.
Major results in 2004 included 1) Studies on the comparison of sites of BGP/MGP gene expression and protein accumulation in adult teleost fishes and during larval development, 2) extend studies on the functional analysis of MGP gene promoter in amphibians and fish, 3) The elucidation of the 3D structure of BGP from a teleost fish with emphasis on its functional implications (comparative studies between fish and mammalian BGPs through protein modeling analysis), 4) Identification of signal transduction pathways involved in the mechanisms of regulation of expression of BGP/MGP genes, 5) Development and characterization of bone- and cartilage-derived cell lines from fish and amphibian, 6) Studies on environmental factors affecting bone biology, 7) Evolution of mineralized tissue through the study of MGP/BGP evolutionary relationship, 8) Identification of genes involved in mineralization using bone-derived cell lines.
Plan for 2005:
Major goals include 1) integrated multidisciplinary approaches to perform *in vivo* / *in vitro* functional analysis of bone/cartilage specific genes through techniques of overexpression and RNA interference, 2) Evolutionary studies on specific gene function, 3) Effect of environmental parameters (pollutants) on bone biology, 4) Development of cell lines from fish with different types of calcified tissues: agnathe (lamprey), cartilaginous fish (shark), marine and freshwater teleost fish (seabream, solea and zebrafish). 5) Development of transgenic zebrafish lines expressing BGP/GFP and MGP/GFP fusion proteins and a line expressing antifreeze proteins. 6) Identification of polymorphisms in specific genes related to environmental adaptations and in populations at risc of developing specific phenotypes.

2. Characterization of the infection of the clam Ruditapes decussatos by the parasite Perkinsus atlanticus. Studies on the biology of the parasite Perkinsus atlanticus and development of new drug therapies.

Main purposes:
Research focus primarily on analysis of parasite-host interactions both in vivo and in vitro. We also porsue the molecular characterization of specific parasite genes involved in host infection and look into the metabolic pathways of the parasite in an effort to develop new drug therapies.
Achieved in 2004:
Major results included the in vitro screening of various drugs for therapy of perkinsiosis and, investigation of their inhibitory effect on a clonal culture of *P. atlanticus*. Identification of purine salvage and shikimate pathways in perkinsus. Developemnt of cDNA libraries focussing on genes involved in host-parasite interactions.
Plan for 2005
Identification of specific genes involved in host-parasite interaction and studies on its regulation of expression through a genome/proteome approach. Identification of environmental parameters capable of modulating parasite growth in vivo and in vitro. Gene expression of the responsible stress genes in *P. atlanticus* according to the presence of different pollutants. Development of a transfection method for *P. atlanticus* cells. In vivo tests in infected *Ruditapes decussates* clams, with drugs capable of acting as new therapy agents.

3. Population conservation studies for the Bonelli's eagle, Hieraaetus fasciatus, and the European otter Lutra lutra.

Main purposes:
Major objectives have been to develop suitable molecular markers for genetic variability studies in order to improve our knowledge on Portuguese populations of two endangered species, the Bonelli's eagle and the coastal otter. The absence of sexual dimorphisms in juvenile eagles makes sexing quite difficult based on morphological parameters only, therefore molecular markers for sexing were also developed. Since eagles are protected species and all handling is very difficult

and stressful for the animal, coupled with the inacessibility of the nests, a method for extracting DNA from feathers was recently developed with sucess. The otters, on the other hand, pose different problems. Being a nocturn species and very difficult to catch, methods for purifying DNA from faeces are currently being optimized in our laboratory, in order to obtain the required biological material to perform genetic analysis.

Achieved in 2004:

For the Bonelli's eagle: The obtention and charaterization of additonal microsatellites for the Bonelli's eagle through analysis of our pre-existing genomic libraries enriched in repeatrd sequences were suitable for an application to a Marie Curie training center in UK. As a result, a total of 19 polymorphic microsatellites were successfully developed and are currently being used to determine degree of polymorphism and initiate genotyping of the population. For otters, DNA from faeces was successfully purified and proven to be from otter using an additional marker from mitochondrial DNA, cytochrom b. DNA was also extracted from tissues collected from dead animals kept frozen or currently in captivity in order to optimize DNA amplification for microsatellite detection and compare it with results obtained from faeces. A monitorization of individuals resulting from faeces collection in the wild was initiated and provided already some information on the genetic variability and effective population size in the coastal area of Southern Portugal.

Plan for 2005:

Method for DNA extraction from feathers will be used to increase our population sampling taking advantage from museum collections (including New York Science Museum and UK Museums). Results from genetic variability and paternity studies of the Bonelli's eagle population from Southern Portugal will be compared with those from populations found in different sites in the rest of the world. Studies on otters will be porsued to extend the area analysed and compare results obtained with those already aquired. We are currently analyzing otter samples for other groups in Portugal as a service, thus putting our expertise in this area to profit, through CCMAR.

Group: Biotechnology and Molecular Biology of Microalgae

Research team

Leader - João Varela
PhD students: Nuno Henriques, Sacha Coesel, and Alexandra Ramos.
Technicians: Ana Rita Marques and Marta Rodrigues

Summary of activities and progress during 2004

Biotechnology and Molecular Biology of Microalgae (BMBM)

Development of transformation procedures for *Dunaliella*

In the previous year, we searched for a chemical agent suitable for selecting positive *D. salina* transformants. We found that the herbicide ammonium glufosinate (Basta) at a concentration of 10 mg/mL was able to abolish cell growth in liquid medium. In order to positively select *D. salina* transformants, we obtained a pCambia plasmid carrying the *bar* gene, referring to Basta resistance. However, this year we found that the same agent was not efficient in reducing the growth of the alga in agar plates. Therefore, it was necessary to test other selective compounds. The antibiotic phleomycin is used for selecting *Phaeodactylum tricornutum* mutants. We found this antibiotic to be also functional for *D. salina*. A concentration of 50 µg / mL at 100 % seawater plates was chosen as optimal condition to select putative *D. salina* transformants.

Development and construction of expression vectors for *Dunaliella salina*

In order for transformed cells to overcome the lethal concentration of phleomycin,we obtained the plasmid pUT58 containing the *ShBle* gene. Our goal was to put this gene under a highly

expressed constitutive homologous promoter and found the β-tubulin promoter suitable. Only recently we succeeded in obtaining the promoter region of the β-tubulin gene. In the mean time, we are working with the promoter and terminator of the well-studied *D. salina Cbr* gene. *Cbr* is not constitutively expressed but is highly up-regulated by environmental stress conditions; a pattern very similar to the *FCP* promoter used for *P. tricornutum* DNA transformation. The *Cbr* promoter and terminator were cloned using PCR techniques on genomic DNA extracted from *D. salina* with primers based on the *Cbr* nucleotide sequence published on GenBank. The identity of these clones was confirmed by DNA sequence analysis. *ShBle* and an additional gene reporter (*Yfp* gene, coding for a protein emitting yellow-fluorescence) was put under the control of the *Cbr* promoter and terminator. *Yfp* provided us with a second control to identify positive transformants.

To introduce these constructs into the cells, we used the Particle Shotgun technique. In this technique, cells are 'bombarded' with particles labeled with DNA (the constructs described above). Two days after bombardment, cells are collected and spread on plates containing phleomycin. Cells displaying a successful integration and expressing ShBle construct in the genomic DNA will be able to resume growth. To find the best experimental setting for introducing foreign DNA into *D. salina*, we tested different parameters. At this moment cells are under selective pressure and the following weeks the first results should come out.

Screening for early and higher expression of carotenoids in mutant and transgenic strains

In the report of last year, we communicated that we had selected several putative carotenoid-overproducing strains of *Dunaliella salina. This year we report that we have selected* 44 cultures of *D. salina*, each derived from a single cell showing: 1) accumulation of higher levels of carotenoids (scored visually), 2) earlier accumulation of carotenoids during the transition between the green and the orange (carotenizing) cell stages, or (3) a different hue as compared with the normal pigmentation of a carotenizing *D. salina* cell. To further verify this phenotype, these 44 cultures of no more than 100 µL were scaled up to 10-ml cultures, and these were then further screened by comparing its growth and pigmentation in cell cultures up to 100 mL. These 44 cultures were then distributed to our collaborators at INETI and Escola Superior de Biotecnologia of Catholic University (ESB-UC) for further analysis.

Each 44 possible mutant strains were further analyzed using the following criteria: 1) growth rate before and during carotenogenesis; (2) total amount of carotenoids per unit volume of cell culture; (3) total amount of carotenoids per cell; and (4) relative amount of specific carotenoids as judged by HPLC analysis of pigment extracts.

From these 44 strains, 3 strains, namely VD1A, VD3A and VD44, were selected for a preliminary analysis of their carotenoid profile. Our collaborators at INETI received VD3A and VD44 strains, whereas ESB-UC received VD1A and VD3A. The reason why VD3A was sent to two different laboratories for further analysis was to verify how reproducible the carotenoid profiles were in diverse laboratory settings.

The results obtained by INETI revealed that the strains VD3A and VD44 did not accumulate significantly higher amounts of total carotenoids. However, when exposed to higher luminosity due to culture dilution and high salt as well as nutrient depletion, VD3A apparently accumulated significantly higher levels of α-carotene per cell as compared to the wild type strain (CCAP 19/30). However, β-carotene levels remained unchanged between the original strain and VD3A. VD44 displayed slightly higher levels of β-carotene during the first 72 hours but failed to accumulate higher levels of these carotenoids as compared with the wild type and VD3A.

As it has been shown that α-carotene possess higher anti-oxidative properties as compared with β-carotene, VD3A was selected for scale-up assays to be carried out by our industrial partner NECTON, S.A.. Although VD3A did not accumulate significantly higher levels of total

carotenoids, the 3-fold increase in α-carotene content observed in this mutant strain (see Fig. 6A), as compared with the wild type strain, was a compelling argument for the initiation of scale-up trials to test the stability of the observed phenotype in outdoors conditions and in larger culture volumes.

Our partner at INETI tested five procedures to induce carotenogenesis with the selected mutant strains:

Method 1 – Nutrient depletion without salt addition in Walne medium at 9% NaCl;
Method 2 – Upshift from Walne Medium at 9 to 35 % NaCl upon nutrient (nitrates) depletion;
Method 3 – Daily gradual upshift from from Walne Medium at 15 to 21, 21 to 28, and 28 to 35% NaCl upon nutrient (nitrates) depletion;
Method 4 - Daily gradual upshift from 15 to 21, 21 to 28, and 28 to 35% NaCl upon nutrient (nitrates) depletion with culture dilution (1:2) with water; and
Method 5 - Nutrient depletion without salt addition at 18% NaCl.

Methods 2 and 4 were found to be inadequate, as cell viability and carotenogenesis were dramatically affected. HLPC analysis of 90%-acetone extracts revealed that the mutant strains VD1A and VD3A displayed similar pigment profiles as compared with the original strain (CCAP19/30), even if subjected to different methods of carotenogenesis induction. Specifically, no significant differences were found between the CCAP and the mutant strains concerning α-carotene, lutein, and 13-*cis*-β-carotene intracellular concentration upon carotenogenesis induction by Methods 1, 3 and 5. However, when the major pigments were quantified by HPLC, VD1A displayed significantly higher levels of *trans*- and *cis*-β-carotene as compared with VD3A and the wild type strain when Method 5 was used to trigger carotenogenesis. The fact that VD3A failed to show any significant differences with respect to α-carotene content when compared with CCAP 19/30 contrasts with the results obtained by INETI. Basically, this discrepancy could be explained either by (1) eventual differences in experimental settings between the two laboratories; or (2) VD3A reverted in ESB-UC's laboratory but not at INETI. The first explanation seems to be supported by the importance of the carotenogenesis induction method employed in the final result. In order to exclude the second possibility, scale-up experiments were needed in order to test the phenotype stability of the VD3A mutant strain.

Development of improved procedures for triggering carotenoid accumulation

At the end of 2003, it was concluded that higher levels of the mRNAs coding for the first two enzymes of the carotenoid biosynthetic pathway (CBP) did not correlate with higher carotenoid intracellular concentration in all experimental settings. This result indicated that additional factors played a role in the massive accumulation of carotenoids in *D. salina* subjected to stress, confirming the importance of the search for novel genes involved in the biosynthesis and accumulation of carotenoids in this alga. During this task, we were able to clone a *D. salina LytB / HDR* homologue, suggesting that carotenoid accumulation may also be regulated in a metabolic pathway upstream of the CBP itself in this microalga. This upstream pathway corresponds to the isoprenoid biosynthetic pathway (also known as methylerythritol pathway), which provides with the substrate (isopentenyl diphosphate, IPP) necessary for the formation of geranylgeranyl diphosphate (GGPP), the substrate of the first CBP enzyme (phytoene synthase, Psy).

Preliminary analysis of *D. salina Hdr* gene expression patterns indicate that this gene is strongly induced upon nutrient stress, but only slightly by high light or salt stress. This contrasts with the strong induction of *Psy* and *Pds* gene expression by high light (see the 2003 year report), suggesting that luminosity is less important for triggering isoprenoid biosynthesis as compared with the induction of carotenoid biosynthesis. Thus, the major common factor for inducing both pathways is nutrient depletion. Although these results are preliminary, they may provide an

important clue as to how *D. salina* regulates the massive accumulation of carotenoids at the periphery of its single chloroplast.

As previously postulated for *Haematococcus pluvialis*, a microalga closely related to *D. salina*, the main function of carotenoids is, most probably, to provide a natural screen for excess of light. This is particularly important for extremophiles, such as *D. salina,* which are able to survive and even thrive in salt ponds. In this habitat, *D. salina* has to withstand extremely high salt concentrations, low levels of nutrients and high insolation. High solar radiation can cause photoinhibition, a mechanism that promotes degradation of photosystem components (*e.g*. D1 protein). If the photosynthetic apparatus is severely damaged, it may be lethal. Therefore, *D. salina* and *H. pluvialis* may protect themselves by inducing the accumulation of a natural radiation filter. However, our results have shown that high light per se may not trigger this massive accumulation of carotenoids in *D. salina.* Nutrient depletion must also be imposed together with high light, a fact well known by algologists, which is supported by our data at the physiological and molecular level.

Taken together, these results suggest that *D. salina* accumulates high amounts of carotenoids by deviating the carbon skeletons produced abundantly by photosynthesis when cells are exposed to high light. The use of these carbon skeletons becomes limited upon depletion of nutrients, such as nitrate, essential for the biosynthesis of amino acids and other cell growth-promoting compounds. Excess of photosynthetic end-products can lead to inhibition of photosynthesis. *D. salina* alleviates this problem by sequestering this excess of carbon in the form of carotenoids and neutral lipid droplets, the latter being the ideal storage place for the pigments. Both can reach very high amounts without affecting the normal metabolism of the cell, as their accumulation does not affect the osmotic potential of the cytoplasm and the plastid stroma. This model explains (1) why there is a need for a two-phase cycle of carotenoid production; and (2) why alternative methods for inducing carotenogenesis (*e.g.* phytohormones and hydrogen peroxide) have largely failed. Therefore, this model give us important clues as to how the triggering of carotenogenesis is regulated and how it can be further optimized.

Plan for 2005

For the year of 2005, the BMBM group will collaborate with Dr. Qin Song from the Instute of Oceanology in China for obtaining stable transformants of *Dunaliella salina.* This collaborator was able to demonstrate transient expression of foreign genes in this alga, but was unable to select for stable transformants. As we have constructed DNA transformation vectors able to confer resistance to the antibiotic phleomycin in this microalga, we expect to isolate stable transformants by selecting them with this antibiotic in collaboration with Dr. Song. This transformation procedure will enable us to finally create gene knock-outs, perform RNAi-based gene expression interference experiments as well as gene overexpression assays. This development will also have a strong impact on the applied research lines we are currently developing together with INETI, ESB-UC and NECTON, S.A.

The year of 2005 will also be a period of transition from work at the level of DNA and RNA to protein. In collaboration with Dr. João Gonçalves, we are developing a method to rapidly screen for interactors of our genes by using the phage display technique. This technology will also allow us to rapidly screen for synthetic antibodies able to recognize our proteins. These antibodies will enable us to study the expression of the genes that seem to play a role in the regulation of the massive carotenogenesis in *D. salina* under abiotic stress, which we have cloned in a previous screen (see report of 2003 for examples of this). This expression studies will be carried out by means of westerm analysis and imunocytochemistry for intracellular localization studies.

We will also continue the characterization of the putative carotenoid-overproducing mutant strains isolated by means of a screen with the carotenoid biosynthesis inhibitor diphenylamine (DPA).

Group: Comparative and Molecular Endocrinology

Research team

Leader(s): Adelino V. M. Canário and Deborah Power
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Undergraduate students: Rita Isabel G. Soares, Vânia F. Martins.

Summary of activities and progress during 2004

The main topics of the group are the molecular mechanisms underlying hormone action and the physiological response of the whole animal. The processes that are the focus of attention are growth and development (with particular emphasis on cartilage, bone and muscle metabolism), reproduction and chemical communication (with recent emphasis on sex determination and pheramones), endocrine disruption in the wild, calcium regulation focussing on hypercalcaemic and hypocalcaemic hormones, and the stress response to normal physiological challenges (with emphasis on ion regulation). An integrated systems approach is being taken and genomics, molecular biology, proteomics, biochemistry, cell biology and whole animal physiology are deployed in order to give an overview of hormone function. The approach encompasses studies of gene regulation, gene expression, post-translational and post-secretory processing, receptor binding, signal transduction and finally the response at a cellular level and also the whole animal response.

Parathyroid hormone-related protein and calcium homeostasis

Mechanisms of ionic calcium homeostasis in fish are different from those in terrestrial vertebrates because there is an almost constant supply of calcium ions from the surrounding water, whilst terrestrial vertebrates rely on dietary sources of calcium, which are very variable and intermittent. In higher vertebrates there are three hypercalcaemic hormones involved in calcium homeostasis, vitamin D, calcitonin and parathyroid hormone (PTH). In fish, the hormones regulating calcium homeostasis are poorly studied and the hypocalcaemic hormone stanniocalcin is assumed to be the principal regulatory factor. In fact PTH has not been identified in fish and the parathyroid gland which produces the hormone in terrestrial vertebrates is absent.
The program of work aims to characterise hypercalcaemic hormones in fish and to establish at the whole organism and cellular level the mechanisms that regulate calcium homeostasis. The organisms studied are a marine teleost, the sea bream (*Sparus auratus*), a euryhaline species tilapia (*Oreochromis mossambicus*) and the model organism fugu (*Takifugu rubripes*) and tetraodon (*Tetraodon nigroverdis*). A new group of PTH-like molecules have been identified and one PTH and one PTH related protein have been shown to be calciotrophic.

Hormonal control of development and growth of fish eggs and larvae

The development of the musculo-skeletal system of the sea bream is being studied and includes the generation of specific tools, the characterization of the developmental expression of skeletal and muscle specific genes and the way in which developmental ontogeny of skeleton and muscle may be altered in abnormal larvae.

1. Egg and larvae are frequently exposed to changing external conditions and the way in which this affects development and the route by which it occurs is fundamental to the understanding of development. The ontogeny of the endocrine system and the effect of environmental factors on development and how it influences directly or indirectly muscle and bone development is being studied (COST925). Integration into a European COST initiative has enriched our approach as it allows technology transfer from mammals to fish.
2. A range of molecular and biochemical markers have been generated including transthyretin a TH binding protein, which may also be an important indice of nutritional status of fish and has potential commercial application (POCTI....). A collaboration to study the influence of nutrition on TH and growth has been initiated with Dr Moutou, University of Thessaly, Greece.
3. Studies are continuing to characterize more fully the structure of genes of interest, such as prolactin (PRL) and its receptor, parathyroid hormone related protein (PTHrP) and a range of G-protein coupled receptors (GPCRs) in order to identify regulatory sequences (eg. promoters) and develop assays to identify factors which influence promoter activity. Significant progress has been made in studying family 2 of GPCRs, which offer an opportunity to study functional modifications which accompanied evolution of duplicate genes. Studies of the ancestral origin of family 2 GPCRs in the metazoan is ongoing and putative receptors have been identified in *C. elegans* and functional studies are underway.

Sea bream genome mapping

The gene map of sea bream has started and this species has been selected as a model organism for the perciformes and in particular for the Sparidae a commercially important group of fish (BRIDGEMAP). The sea bream has a genome (0.8pg/haploid nuclei) which is only slightly larger than that of Fugu. This year the target of 500-1000 expressed sequence tags (ESTs) was passed and a total of 3000 were generated and represent an important resource for future work and for the scientific community at large. A sea bream radiation hybrid is available and 250 markers have been mapped. The methodology for this process has been fully developed and currently a further 400 markers are being mapped. In addition, 150 microsatellites have been developed and their polymorphism determined, over 50 are within candidate genes for a number of traits. An initial linkage map has been generated for future for QTL analysis.

Steroid receptor expression and function

The estrogen receptor (ER) is a transcription factor of the nuclear receptor family with a wide range of functions in vertebrates. There is growing evidence that estrogens play important roles in both normal and xenoestrogen disrupted testis physiology. However, the mechanisms and genes involved, in particular in fish, remain largely unclear. By subtractive hybridization, we have isolated 152 different candidate estrogen-responsive genes in the testis of male sea bream The E_2-up regulation of some of these genes (choriogenin L and H, vitellogenin I and II, apolipoprotein A-I, fibrinogen β and γ and thyroid receptor interacting protein 4) was confirmed by semi-quantitative RT-PCR in testis cDNA of individual E_2-treated fish. Most of these genes are typical E_2-induced genes in liver.

Control of sexual determination and differentiation

Fish have a variety of sex determining mechanisms, including environmental sex determination. However, the genes responsible for sex determination are largely unknown. We have isolated a number of genes which are implicated in sex determination in mammals and are analysing their expression patterns in fish. Dax-1 is one such genes which is under close scrutinity. Current studies on this gene include promoter regulation and overexpression in vivo.

Fish Chemical Senses: identification of active compounds and modes of action

The understanding of olfaction in fish is fundamental to investigations into chemical communication, reproduction, ion-homeostasis and feeding. Although the importance of pheromones in fish reproduction is well recognised, given the phylogenic diversity and the wide range of habitats and reproductive strategies used by teleosts, very few species have been studied

in detail, the model species being the goldfish. The main impediment in this respect is the lack of knowledge of the chemical identity of the active compounds involved. This is particularly true of the cichlids and blennids, especially considering their highly developed mating systems and use of parental care. In addition, many exploited fish species are marine (*e.g*. *Sparus aurata*, *Solea senegalensis*), but most studies by other laboratories have chiefly focused on freshwater species. In marine species, very little is known about the potential role of pheromones in reproduction, and more detailed knowledge would facilitate informed and efficient management of broodstock. Both marine and freshwater species have been object of research by the research team; the peacock blenny (*Salaria pavo*), the gilthead seabream (*Sparus aurata*), the Senegal Sole (*Solea senegalensis*), the goldfish (*Carassius auratus*), the Mozambique tilapia (*Oreochromis mossambicus*), the tench (*Tinca tinca*) and the eel (*Anguilla anguilla*).
During the breeding season, *S. pavo* males develop an androgen-dependent anal gland (AG) from the first two rays of the anal fin. Behavioural experiments have shown that a putative pheromone from the AG promotes female attraction to nesting sites and influences female mate choice, thereby affecting male reproductive success. However, this putative pheromone is not involved in sex recognition, since male visual cues are sufficient to trigger female courtship behaviour. This is the first demonstration of an external structure specialised in the production/release of sex pheromones in teleosts. This year research focused on on the establishment of electrophysiological methods to test fractions obtained from S pavo holding water fractionated by hplc and comparisons to the freshwater S fluviatilis. The results have been presented in national and international scientific meetings, have contributed to a Master thesis, and will be submitted for publication in 2005.
O. mossambicus is a maternal, mouth-brooding cichlid; territorial males group in "leks", and are actively sought out by reproductive females. After the female has chosen her mate, spawning takes place and the female picks up the fertilized eggs in her mouth and incubates them, until they hatch, in solitude. Unique HPLC fractions of urine from territorial males were shown to activate olfactory receptor neurones in females. These fractions were isolated in collaboration with Prof. Peter Sorensen (Univ. Minnesota, USA) The molecular weight of a putative pheromone has been determined.. In addition, HPLC fractionation of extracts of water conditioned by females and screening of their olfactory potency via EOG recordings on males. Some results have been published (Almeida *et al*., 2005, Miranda *et al*., 2005); others will be submitted for publication in 2005. In a related project, the possible role of chemical communication in the reproduction of the eel (*Anguilla anguilla*) was investigated in collaboration with Joan Cerdà and Mar Huertas from IRTA, Tarragona, Spain. Preliminary results have shown that eels have high olfactory sensitivity to conspecific bile and mucus. Furthermore, the active compounds differ according to sex and/or state of maturation. Not only is this good supporting evidence for a role for chemical communication in a species that spawns in seawater, but it is the first time that such olfactory sensitivity to mucus has been demonstrated in a quantitative manner.

Research on the role of olfaction in the feeding behaviour of *Solea senegalensis* aiming at the identification of attractants released by natural food organisms has made important progress towards the identification of specific odours present in prey, using behaviour and electrophysiological assays. This work is being carried out in collaboration with Joerg Hardege, Univeristy of Hull, UK. The epithelium of the upper was significantly more sensitive to the aliphatic amino acids L-alanine, L-glycine, L-threonine and L-serine than the lower epithelium. The lower epithelium was significantly more sensitive to aromatic amino acids such as L-tryptophan, L-tyrosine and L-phenylalanine. Both epithelia had similar sensitivity to basic amino acids (L-arginine and L-lysine) and sulphur-containing amino acids L-cysteine and L-methionine. Neither side was sensitive to acidic amino acids (L-aspartate and L-glutamate) nor the D-isomers of any amino acid tested. The upper side was much more sensitive to conspecific-derived stimuli (bile and intestinal fluid) than the lower side. We suggest that these differences in sensitivity are related to different functional roles in the location (upper) and identification (lower) of potential food items; the upper olfactory epithelium is likely to be more involved with chemical communication than the lower (Velez *et al*., in press).

Plan for 2005

Parathyroid hormone-related protein and calcium homeostasis

A range of physiological and molecular studies are being carried out to elucidate the role of PTH-like molecules in reproduction, skeletal development, calcium balance and immune response in sea bream and other teleosts. The role of other calcium regulating hormones in these processes will also be studied as will their interaction with PTH/PTHrP. The approaches include 1) the mechanisms through which PTHrP regulates calcium balance in fish using Ussing chambers and cell culture (enterocyte and gill mitochondria rich cells); 2) receptor characterization and PTHrP responsive genes using subtractive hybridization and microarrays; 3) identification of PTHrP responsive tissues using bioassays and biochemical assays; 4) bioactivity and post-translational and post-secretary processing of the diverse PTH and PTHrP proteins identified in fish; 5) in situ hybridisation to establish cellular location and ontogeny of PTH/PTHrP and responsive genes in fish; 5) Knock-down models to establish the importance of PTHrP responsive genes.

Hormonal control of development and growth of fish eggs and larvae

Developmental studies have largely focused on the genes and proteins involved in the formation of the embryo. The role of endocrine factors in these and subsequent developmental processes has largely been neglected, particularly in metamorphosis. Considerable discussion exists about the significance of metamorphosis in round and flat fish. The way in which endocrine factors and in particular the thyroid and pituitary axis control this process is still not well established and will be the object of study:

1. The major morphological transformations of round and flat fish will be compared during metamorphosis, using external morphology and internal morphology (whole mount and sections). 2. Development and application of molecular and biochemical markers of key physiological systems, nervous system, digestive system, musculo-skeletal system, endocrine system in round fish and flat fish. Comparison of marker ontogeny in round and flat fish. 3. Experiments to establish the effect of absence of excess of hormone on morphological markers of metamorphosis and molecular/biochemical markers. Correlation of markers with metamorphic progression. 4. Potential application of the results to the industry. The results of the preceding studies will be considered in the perspective of their implementation as a means of quality control in the Industry.

Diet has an important influence on normal development and growth, the role of thyroid hormones in the this process is undisputed. However, relatively few studies of the role of thyroid hormone binding proteins which regulate free hormone availability have been conducted. The role of transthyretin (TTR) in transporting TH in fish and its use as an indicator of health will be studied.

Molecular evolution of hormones and receptors

The genome of the model species *Fugu rubripes*, a teleost with a remarkably small genome (400Mb), was fully sequenced in 2001 and of the related *Tetraodon nigrovirdis* in 2004. These resources have opened the door for comparative studies of hormones and receptors in Fugu and tetraodon and other teleost fishes. Several models of genome evolution exist, the most popular suggests that 2 rounds of whole genome duplication occurred before the emergence of the jawless fish and that subsequently in the teleost lineage a further round of duplication occurred. The persistence of duplicated hormone and receptor genes in the genome of the Fugu will be studied in the secretin family of G-protein coupled receptors in order to establish mutation rates and the reason that duplicate genes have persisted. In parallel the corresponding cDNA for these receptors will be isolated in the sea bream to establish transcript number and the existence of splice variants. This will entail completion of work already initiated in *Fugu* to fully characterise the genes in the secretin family of G-protein coupled receptors. Extensive analysis *in silico* of the genes from Fugu and available sequences from representatives of other taxa will be carried out. Functional studies to establish the affinity of ligands for duplicate receptors will be performed. The approach taken will

form the basis of future studies to establish the cross-talk which occurs between gene evolution and function of other hormone and receptor genes. In addition, the hypothesis that receptors arose first and then adopted one or more ligand in metazoa will be tested. A more applied aspect of the work will be initiated, that of biopanning in order to isolate novel ligands from marine organisms.

Sea bream genome mapping

1. Implementation of genotyping in a commercial aquaculture company in order to characterise the broodstock. 2. Physiological studies will be initiated in the context of a European project to establish the heretability of stress and disease resistance. Experiments will be conducted and sample analysis initiated. 3. Mapping of genes on the sea bream radiation hybrid will be continued in order to place up to 1000 markers on the map. 4. Linkage analysis will be conducted with candidate genes to establish their weight in QTL.

Control of sexual determination and differentiation

Work will concentrate on developing a medaka transgenic model overexpressing DAX-1, the analysis of gene promoter regulation and the comparative analysis of sex determination gene expression in different species..

Steroid receptors and development of biomarkers of endocrine disruption

Soe of the tools developed in this project will be the basis for a start up company to be setup. During 2005 the analysis of samples to screen for signs of endocrine disruption in Portuguese estuaries should be finalized. The assays for TH disrupting activity will be validated and assays for androgen disruption will be established.

Fish Chemical Senses: identification of active compounds and modes of action

1) Determination of how many odorants (putative pheromones) are present in the urine of territorial and non-territorial male tilapia (*O. mossambicus*) and comparison of their concentrations. This will be achieved by combining HPLC fractionation and EOG recordings for screening the olfactory potency of HPLC fractions. The results of this work in collaboration with the team lead by Prof. Peter Sorensen (Univ. Minnesota) will be the ground for tentative chemical identification of the putative male pheromones. In addition, a behavioural and/or physiological assay should be developed to test the effect of the putative pheromone on females.
2) Investigate the role of chemical communication in aggressive interactions between males of *O. mossambicus*.
3) Continue the collaborationwith the research team lead by Prof. John Pickett at Rothamsted Research aiming at the chemical identification of the putative pheromone from the anal gland of male *S. pavo*. The behavioural effect of extracts of water conditioned by males, of anal glands and of identified compounds (if made available) will be tested.
4) Continue the collaboration with Prof. John Pickett *et al*. aiming the tentative identification of putative pheromones released by female tilapia (POCTI/BIA-BDE/55463/2004).
5) Initiate a comparative study of the pheromonal system in two related species of blennies: “Identification of Sex Pheromones from the Anal Gland of Male Blennies, *Salaria pavo* and *S. fluviatilis* (Pisces: Blenniidae)”; ref. POCTI/BSE/45843/2002.
6) Investigate if, and how, olfactory detection of calcium and sodium in the environment influences the neural and neuroendocrine pathways regulating ion homeostasis. These studies will initially focus on the putative role of the crypt cell olfactory receptor neurones by a combination of electrophysiological and cell-labelling techniques (POCTI/BIA-BCM/55467/2004 and POCTI/BIA-BCM/60554/2004).
7) Continue investigations aiming at the identification of attractants associated with natural sources of food of the Senegal sole (*Solea senegalensis*). This will be carried out in collaboration with Prof. Joerg Hardege (Univ. Hull, U.K.) under a Treaty of Windsor programme (ref. B-71/04).

8) Initiate investigations into the olfactory transduction process of marine teleosts, particularly where and how they differ from freshwater fish with respect to the use of external ions.

Group: Physiopathology

Research team

Leader: Josefina Coucelo
Post-doc: Natércia Joaquim
PhD students: Gisela Borges; Sandra Soares
Undergraduate students: Rita Bicho, João Alves, Cláudia Pereira

Summary of activities and progress during 2004

1- Cardiac Performance in the Atlantic Wolffish (Anarhichas lupus) Following Acute Changes in Temperature and Oxygen *(collaboration with the Oceans Science Centre – Memorial*
An *in vivo* study was performed to examine the effect of increased environmental temperature and hypoxia on the cardiac performance of the Atlantic wolffish (A*narhichas lupus*). These environmental factors are known to strongly affect the cardiac physiology of pelagic (active) fish species, but few studies have been conducted on benthic (sedentary) marine fishes. Further, although several species of wolffish in the North Atlantic are listed as endangered and A*narhichas lupus* has been identified as a species with great aquaculture potential, their physiology is virtually unknown.
We exposed 8^{o}C acclimated wolfish to an acute temperature challenge (6 to 16^{o}C at 2^{o}C h^{-1}) and graded hypoxia (final O_2 level 20% sat.), and measured cardiac function (cardiac output, Q; heart rate, HR; and stoke volume, SV) using Transonic® flow probes.
Our results showed that the Atlantic wolffish modulates HR, but not SV, in response to acute changes in oxygen and temperature, and that this results in an 'atypical' Q response (decrease) in the face of decreased water oxygen levels. Whether cardiovascular responses to other challenges (e.g. exercise, stress) are different from those reported for other teleost species is unknown, as is whether the lack of an increase in SV (to defend Q) during hypoxia is characteristic of hypoxia-tolerant species.

2- Physiopathological responses to toxic metals intoxication (collaboration with FCT, UAlg and Universidad de Extremadura, Badajoz)
Vanadium is a heavy metal with increased environmental circulation, resulting from various anthropogenic activities and it is of great concern due to its toxicity and accumulative behaviour at specific target organs, such as the liver and kidney, inducing oxidative damage, lipid peroxidation and changes in haematological, reproductive and respiratory systems. The study of vanadium (and different vanadate oligomeric species) toxicity is of great concern to well understanding of the biochemical effects of this metal. Specially, due to the duality of effects that are attributed to vanadium. Despite its toxicity at higher concentrations, vanadium is assumed to be an essential element for several organisms, inducing a wide range of beneficial effects (being the insulin-mimetic action the more relevant). Although, its biological role is far from a clear identification. Besides it is known that, vanadate species are known to interact with many proteins besides affecting biological mechanisms; i*n vivo* toxicological studies of vanadate oligomers effects are scarce.
During the last year, we further explored the oxidative stress responses induced by an acute exposure to a sub-lethal concentration of two vanadium solutions (metavanadate and decavanadate) administrated intravenously, on the cardiac muscle of the marine teleost *Sparus aurata* (gilthead seabream).
In 2004, *in vivo* studies suggested that an acute exposure to different vanadate oligomeric species promotes different vanadium distributions, in blood and heart of *S. aurata*. Furthermore, cardiac mitochondria seem to be a target of vanadium accumulation.

Besides vanadium accumulation had produce an increase in the overall ROS production in cardiac tissue, upon metavanadate intravenous injection, it was not observed lipid peroxidation neither changes in antioxidant enzymes activity.
These preliminary studies allow considering the cardiac mitochondria as a model to oxidative stresse evaluation induced by different vanadate oligomeric species, at a subcellular level. It was also concluded that, metavanadate affects the mitochondrial functional integrity, decreasing significantly the F_1,F_0-ATPase activity.

3- Endothelial dysfunction and cardiovascular diseases (collaboration with Hospital do Barlavento Algarvio)
Hipertension, diabetes and atherosclerosis are complex and progressive conditions that share several common antecedents. Endothelial dysfunction, which is implicated in the developing of several cardiovascular diseses, has been often associated with the existence of oxidative stress. Oxygen free radicals are produced in aerobic organisms during mithocondrial physiologic or physiopathologic oxidative metabolism. These radicals are able to react with various types of molecules, including lipids, carbohydrates, proteins, nucleic acids and connective tissue macromolecules, thus interfering with cellular function. When these reactive agents are not rapidly eliminated they are capable of producing cellular and tissue lesions, including vascular lesions. The toxicity of oxygen free radicals in living tissues is well established and is implicated in the etiology of a number of disorders, including diabetes mellitus. However, studies involving antioxidant enzymes are contradictory since they describe increased or diminished activities of superoxide dismutase, catalase and glutathione peroxidase in the blood of diabetic patients.
The aim of this work is to study oxidative stress and endothelium dysfunction mechanisms in hypertensive and diabetic patients, and in experimental models. During 2004, hipertensive patients and controls subjects were referred and blood samples were collected in order to determine: metabolic status (glucose and cholesterol content in serum), cardiovascular risk indicators (C-reactive protein, homocysteine, creatinine), antioxidant enzymes activities, lipid peroxidation products. Coagulation parameters and cardiovascular risk parameters were found to be altered in hypertensive patients: fibrinogen and homocysteine showed to be higher in these patients. Cardiovascular risk and inflammation was associated with altered oxidative stress parameters. Lipid peroxidation was higher in hypertensive patients and antioxidant enzymes (catalase, superoxide dismutase and selenium-dependent gluthatione peroxidase) were also augmented. Our results confirm that hypertensive patients are exposed to both oxidative stress and cardiovascular risk; although these features seem not to be quantatively correlated.

Plan for 2005

It is our purpose to continue the work developed during the past years, specifically:

Oxidative stress induced by metal ions in cardiac muscle (collaboration with FCT, UAlg and Universidad de Extremadura, Badajoz)

1) Antioxidant enzymes activities, lipid peroxidation products and glutathione content measurements in *Sparus aurata* (gilthead seabream) cardiac muscle from control and vanadate intoxicated individuals;
2) Metal subcellular distribution studies in fish blood and cardiac tissue from control and vanadate intoxicated individuals;
3) SERCA-2 activity in cardiac homogenates from control and vanadate intoxicated fish;
4) Analysis of an environmental pollution marker and a cardiovascular risk indicator (C-reactive protein) in fish plasma from control and vanadate intoxicated individuals;
5) Mitochondrial oxygen consumption monitorization after *in vitro* incubation with vanadate;
6) Mitochondrial membrane potential, ROS production and F_1,F_0-ATPase activity assays, in cardiac and hepatic mitochondria of fish and rat, after *in vitro* incubation with vanadate;
7) Neonatal rat cardiomyocytes primary cultures setup to vanadium exposition studies;
8) Viability, membrane potential, ROS production and calcium homeostasis studies in primary cultures of neonatal rat cardiomyocytes treated with vanadate;

9) Identification and quantification by Nuclear Magnetic Resonance (NMR) of vanadate oligomeric species present in the vanadate solutions used in this study;
10) Monitorization of vanadate reduction to vanady by EPR, in the conditions of the previous studies.

Accumulation of heavy metals and levels of antioxidant enzymes in the Arabian Sea, Sultanate of Oman (collaboration with the College of Sciences of the Sultan Qaboos University and the Ministry of Regional Municipalities, Environment and Water Resources of Oman)
In a world wide scale, populations of marine turtles are in decline. All species of sea turtles are included in the list of the red book of the IUCN. The marine turtle *Chelonia mydas,* commonly named green turtle, is endangered in several areas of the world. The biggest nesting population of green turtle of the Indic Ocean is in the natural reserve of Ras Al Hadd (Sultanate of Oman, Arabian Sea).
Marine pollution caused by plastics, heavy metals and PCBs has been of great concern and it is possibly implicated in the decline of marine turtles populations. Heavy metals are carcinogenics and genotoxics. Furthermore, many metals act in cells by producing reactive oxygen species (ROS), thus causing oxidative stress. Considering this, it is our aim to study oxidative stress as a pollution indicator in a population of green turtles (*Chelonia mydas*) of the Arabian Sea. Therefore, in 2005, we will conduct the following studies:
1) Analysis of the heavy metals concentrations in the blood and eggs of nesting green turtles;
2) Analysis of the heavy metals concentrations in the liver of green turtles (this study is only possible when turtles are found dead in the beach);
3) Determination of antioxidant enzymes (catalase, superoxide dismutase, total gluthatione peroxidase and Se-dependent gluthatione peroxidase) in the erythrocytes of nesting green turtles;
4) Study of lipid peroxidation in the plasma of nesting green turles;
5) Determination of ROS production in the erythrocytes of nesting specimens of *C. mydas*.

Effects of vanadate in the activity of Ca2+-ATPase (SERCA2) in the ventricle of Sparus aurata
Cardiac sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2) plays a major role during excitation-contraction coupling transporting Ca^{2+} from cytosol into the lumen of the sarcoplasmic reticulum and thus promoting the relaxation of the muscle.
In contrast to the efforts to quantitatively assess and characterize transsarcolemmal Ca^{2+} fluxes in the cardiac muscle of fish, few studies have directly measured sarco/endoplasmic reticulum Ca^{2+}-ATPase activity and Ca^{2+} uptake.
Vanadium is a world wide spread heavy metal that results from anthropogenic activities and it is of great concern due to its toxicity in several organs. However, it has been also reported that vanadate reverses drug resistance, increases glucose metabolism and inhibits or stimulates the activity of several enzymes. Despite its toxicity at high concentrations, vanadium is assumed to be an essential element for organisms, although its biological role is not clear. In cells, oxovanadium (IV) species are essentially the vanadium species present, even though some of the interest in vanadium biochemistry is due to the similarities between the phosphate and vanadate chemistries in solution. In that sense, a good number of the biochemical importance of vanadium is associated with the +5 oxidation state (vanadate). However, in vanadium (+5) solutions diferent oligomeric species can occur simultaneously in equilibrium such as monomeric (V1), dimeric (V2), tetrameric (V4) and decameric (V10) species. These vanadate species are known to interact with many proteins besides affecting biological mechanisms; in fact, vanadate oligomers are known to interact with the proton ejection by the Ca^{2+} pump of the sarcoplasmic reticulum. Hence, we propose to evaluate the effects of different oligomeric species of vanadate in the process of excitation-contraction coupling of the cardiac tissue, by studying the activity of Ca^{2+}-ATPase in the ventricle of a fish (*Sparus aurata*). The following studies will be conducted:

1) Evaluation of the stability of metavanadate and decavanadate (1mM of total vanadium) in the presence of sarcoplasmic reticulum vesicles isolated from the ventricle of *S. aurata* studies;
2) Determination of the activity of Ca^{2+}-ATPase from the ventricle of *S. aurata* exposed to metavanate (1mM) and decavanate (1mM) during 1, 3 and 8 days.
3) Determination of the activity of Ca^{2+}-ATPase from the ventricle of *S. aurata* exposed *in vitro* to several concentrations (50 μM-1mM) of metavanate and decavanate.

Endothelial dysfunction and cardiovascular diseases

1) Induction of atherosclerosis in New Zealand rabbits by feeding the animals with a cholesterol-rich diet (0,5%) during 8 weeks;
2) Treatment of a sub-group of atherosclerotic rabbits with rosuvastatin during 6 weeks;
3) Study of the coronary microcirculation of atherosclerotic rabbits, atherosclerotic rabbits treated with rosuvastatin and control rabbits through contrast echocardiography, in order to study the myocardial perfusion;
4) Study of antioxidant enzymes activities (catalase, superoxide dismutase, total gluthatione peroxidase and Se-dependent gluthatione peroxidase) in the erythrocytes of atherosclerotic rabbits, atherosclerotic rabbits treated with rosuvastatin and control subjects;
5) Lipid peroxidation products analysis in the plasma of atherosclerotic rabbits, atherosclerotic rabbits treated with rosuvastatin and control subjects;
6) ROS production in the erythrocytes of atherosclerotic rabbits, atherosclerotic rabbits treated with rosuvastatin and control subjects;
7) Study of angiotensin-converting enzyme in the plasma of atherosclerotic rabbits, atherosclerotic rabbits treated with rosuvastatin and control subjects;
8) Evaluation of the metabolic status (glucose and total cholesterol, HDL cholesterol and LDL cholesterol content in serum) of atherosclerotic rabbits, atherosclerotic rabbits treated with rosuvastatin and control subjects;
9) Evaluation of cardiovascular risk indicators (C-reactive protein, homocysteine, creatinine) in the blood of atherosclerotic rabbits, atherosclerotic rabbits treated with rosuvastatin and control subjects;
10) Histological evaluation of possible damaged areas in the left ventricle myocardium of atherosclerotic rabbits and of atherosclerotic rabbits treated with rosuvastatin.

Group: Biophysics

Leader - Leonor Cruzeiro-Hansson
Paulo Silva, PhD Student

Summary of activities and progress during 2004

The work on protein folding and function is being pursued in two main directions: 1) is the simulation of vibrational energy transfer in specific proteins, such as the F1 F0 ATP synthase, myoglobin and prions. The aim is to establish, both theoretically and experimentally, that vibrational energy transfer is an important step in the way proteins works. 2) A second direction is the derivation of a model that can describe the transfer of vibrational energy to the conformational degrees of freedom. Paulo Silva is working along this line and has derived a set of equations that can be applied to the dynamics of a large variety of models. Its application to a specific case has already led to one publication and four communications in international meetings.

Plan for 2005

New studies of protein structur will be carried from the multi-funnil point of view, i.e., the notion that proteins can assume diffferent structures. More general research on non-linear networks will be carried out in collaboration with Prof. Chris Eilbeck.

Group: Aquaculture

Research team

Leader: Maria Teresa Dinis
Researchers: Luis Conceição
Post-docs: Florbela Soares, Pavlos Makridis, Laura Ribeiro, Neil Ruane, Cláudia Aragão, Nuno Simões
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Undergraduate students: Ana Couto, Ricardo Costa, Luís Viana, Rita Costa, Ana Teresa Gonçalves, Cláudio Torres.
Aquaculture technicians: Helena Damásio, Helena Teixeira

Summary of activities and progress during 2004

The central aim of the group is to contribute to the sustainable development of the aquaculture industry, through basic and applied research directed to the optimisation of cultivation techniques and to the bottlenecks identified in the cultivation of new species for aquaculture. Ongoing projects involve nutrition physiology, morphology, stress physiology and microbiology studies, in particular during the early stages of marine fish. In 2004 research was also performed on broodstock management, and zootechnical aspects of larval and juvenile rearing of sole (*Solea senegalensis*), seabream (*Sparus aurata*), red porgy (*Pagrus pagrus)* and grouper (*Epinephelus marginatus*), as part of the research projects where the group is involved.

Effect of dietary neutral lipid level and source on food intake, digestion and growth in marine fish larvae

The requirements for essential fatty acids (EFA) have been extensively studied in marine fish larvae but few studies have examined quantitative lipid needs in larval diets. The attempt to meet larval requirements using poor sources of EFA (predominantly neutral lipids) may result in excessive lipid content and a number of authors have reported poor larval growth and performance associated with a high lipid content of the diet. Studies were carried out on commercially valuable farmed species (Solea senegalensis, Sparus aurata and Dicentrarchus labrax), to investigate the effects of total neutral lipid level and lipid source (i.e., FA composition) on key factors involved in larval growth. Significant effects were observed for FA and amino acid absorption efficiency and metabolism, food intake, lipase activity, as well as gut tissue structure.
It seems that lipid level in diets for marine fish larvae may have an important impact in several factors influencing larval growth and development. However, dietary lipid quantitative supply cannot be dissociated of its FA composition, which appears to play a central and determinant role on the nutritional and physiological impacts (at the ingestion, digestion and absorption levels) of dietary lipid inclusion. The data obtained are still being further treated.

The effect of green water on larval development

During this period the influence of different microalgae (Tetraselmis chuii, Isochrysis galbana, Phytobloom) on marine fish larvae (Senegalese sole and Gilthead seabream) development was studied focusing on aspects like digestive physiology, intermediary metabolism, water quality, food ingestion, and as probiotcs.
The presence of microalgae in the rearing water resulted in higher survival rates when compared with clear water treatment. However no significant effect of microalgae could be established in terms of the digestive enzymes actvity, alhtough there was a tendency that fish larvae reared with microalgae exhibited a better digestive capacity that could be important in later development.

Both lipogenic enzymes and amino acid metabolic enzymes in seabream larvae were not affected with different microalgae. Further reaserch is needed concerning the nutritional composition of life food maintained with different microalgae.
A higher food intake of live food was observed when fish larvae were maintained with microalgae. Among the microalgae used *Tetraselmis chuii* presented the best result.
Microalgae have been pointed out as an important vehicle to maintain water quality in terms of ammonia. Water samples for ammonia analysis were collected during larval rearing with different microalgae.
In collaboration with an ongoing project of this research group, bacteria strain isolated from microalgae with ability to compete with pathogens was used during larval rearing. The purpose of this experiment was to observe survival and growth rates when fish were reared from mouth opening with this bacteria strain, being the other treatements used clear water and microalgae supernatant. Bacteria and microalgae supernatant treatments presented similar survival and growth results when compared with clear water, suggesting that microalgae might be a source of benefical bacteria strains.

Modelling the utilisation of dietary amino acids in fish larvae
Further improvement of growth performance in fish larviculture is closely linked to a better understanding of the dietary amino acid (AA) requirements, and therefore of the processes involved in AA metabolism. In recent years major advances in the understanding of fish larvae amino acid metabolism have been accomplished, in particular through the use of tracer studies. However, interpretation of information from tracer studies is usually limited to the comparison of a number of body compartments in a few time points. Modelling is a holistic approach to integrate knowledge on growth and metabolism and identify gaps .A dynamic mechanistic model that simulates AA metabolism of fish larvae was developed using the POWERSIM package. Its objective is to improve the understanding of larval digestion and absorption of dietary AA, and the postprandial AA metabolism and growth. The model should also assist in the interpretation of results obtained using tracer studies. The model is driven by amino acid intake, with the absorbed dietary AA being used for energy production or for biosynthetic processes. The first version of the model was so far tested with Senegalese sole (Solea senegalensis) fed Artemia, and was parameterized using literature data. The model allows to integrate the results obtained after feeding a single meal with tracer AA, and following these tracer AA in the free AA and protein pools of larval gut and larval body at different time points after the meal. Model simulations permit to study the dynamics of the changes in the larval free AA and protein pools. Calibration of this dynamic model using tracer studies data also allow to calculate the instantaneous rates of different processes involved in AA metabolism: gut AA catabolism, gut protein synthesis, gut AA evacuation, transfer of AA from the gut to the body, body protein synthesis and body AA catabolism. Due to its mechanistic nature, the present model can be used with different AA tracers, and also for other fish species. Work is in progress on these aspects and in further refinement of the model. (Work in collaboration with Prof. Ivar Ronnestad, Univ. Bergen, Norway)

Health management in sole rearing
The work on use of probiotic bacteria and immunostimulants during the rearing of Senegalese sole has been continued. Intestinal bacteria in marine fish may produce antimicrobial substances which inhibit pathogenic bacteria. The influence of diet on the antimicrobial activity of bacterial strains isolated from the gut microflora of Senegalese sole, Solea senegalensis, was determined. Pre-adult 15 month old fish previously fed on an artificial diet, were fed polychaetes (Hediste diversicolor), which form part of the natural diet of Senegalese sole. Samples were taken 0, 3, and 6 weeks after start of the experiment from the stomach, small, and large intestine of the fish. The bacterial strains isolated from these samples were pure cultured and several biochemical tests were run. Amplification and sequencing of 16s rDNA fragments were used to identify the majority of the bacterial strains isolated. Feeding with polychaetes significantly increased the numbers of presumptive Vibrios in the gut. The antimicrobial activity of the bacterial strains isolated, as determined by two in vitro approaches, was significantly increased by feeding with polychaetes.

Pigmentation abnormalities in sole

A major problem with flatfish culture is the occurrence of malpigmentation. Although recent advances in nutrition and zootechnical aspects have alleviated the problem somewhat, the underlying mechanisms as to the cause are still unknown. A series of experiments were carried out to examine the influence of tank colour (white, yellow, black) on the physiology of the sole. All fish changed their body colour to match the surrounding environment. Adaptation to black tanks (five weeks) increased basal cortisol levels and induced facilitation of the cortisol response to stress. However, levels of α-melanophore-stimulating hormone (α-MSH) were lower in black adapted fish. In addition, *in vitro* studies showed that α-MSH release from the pituitaries of black adapted fish were also lower indicating inhibition of hormone synthesis. Although this may raise the question of what role α-MSH plays in background adaptation in sole, we have found that addition of α-MSH can result in melanophore dispersal in isolated scale slips. This dispersal would appear to be mediated through the secondary messenger cAMP pathway suggesting the presence of α-MSH receptors in scale cells.

Nutritional effects on fish proteome expression

Preliminary work was conducted on the effect of nutrition and feeding on fish proteome expression. Groups of zebrafish were fed diets at three different ration levels. The analytical work was performed at the Proteomic Core Facility at European Molecular Biology Laboratory (EMBL) in Heidelberg. The 2D-PAGE- based analysis demonstrated clear differences between the treatments and a differential expression of proteins in the dissected carcasses (muscle). The protein spots were excised and analysed on a MALDI TOF and QTof. The results from the preliminary study showed that growth rate strongly affected the relative proteins expression in the muscle: annexin 6 was upregulated in fast growth while there was a relative downregulation of actin gamma, a protein similar to proteasome (prosome, macropain) 26S subunit, type 1 and 2 cytokeratin, and keratin 18. Under starvation fast skeletal myosin heavy chain 4, actin I and alpha tropomyosin were all regulated at higher levels than the rest of the identified proteins. Some of the protein spots in the fast growing fish could not be identified with peptide mass fingerprinting and some were unknown proteins (previously only predicted by genome expression prediction or cDNA open reading frames). This study demonstrates the skeletal muscle plasticity in fish with different growth rates and during starvation but also points to potentially important spin off results regarding knowledge for the quality assessment of fish: i.e. taste, texture and allergenic properties.
(Work in collaboration with Prof. Ivar Ronnestad, Univ. Bergen, Norway)

Broodstock management

The objectives for 2004 were the identification of the parameters responsible for sole maturation and reproduction, one important bottleneck on sole cultivation. Spawnings with good quality eggs were obtained from two groups, kept under natural photoperiod and temperature. The temperature was identified as one of the triggering environmental parameters for the maturation and spawning of sole broodstock.

The spawning performance of the 4 groups in captivity was evaluated based on the egg quality and egg hatchability.

In october 2004 a new group of male sole broodstock was established. All fish were tagged and sex determined. Sperm extraction was performed every 15 days to analyse sperm quality in fluent males before, during and after apawing season. Seminal plasma, osmolarity, pH, spermatozoa motility andcell concentration were the parameters choosen to characterize sperm quality.

The existing four groups of fish were maintained in a open system at the Experimental Station of Ramalhete. In October 2004 two groups were set feeding with squid and worms and the remaining groups were fed squid and mussels.

A dusky grouper broodstock is being kept in collaboration with IPIMAR/CripSul, at their EPPO facilitity. Fish were canulated in summer 2005, and hormonal induction of spawning in grouper using GnRHa slow-releasing devices was attempted. No successful spawnings were however obtained. These results suggest that the administration of the GnRHa slow-releasing devices was should have been done a few weeks earlier in the annual maturation cycle.

Zootechnical improvements in the larval rearing of new species for Aquaculture

Studies were conducted in the optimization of the larval rearing conditions and feeding regimes of sole (*Solea senegalensis*), red porgy (*Pagrus pagrus)* and grouper (*Epinephelus marginatus*).
Weaning is one of the traditional bottlenecks in sole culture. In continuation of studies from the previous years at CCMAR, additional experimental diets and weaning strategies for sole were tested together with the study of sole digestive capacity. Early weaning seems to be determined more by size than age. Fair results are obtained with initial wet weights 40-60mg. The survival rates during weaning are still highly variable ranging from 15% to 94% in comparable conditions. Senegalese sole can now be weaned with available commercial diets, but a consistent high survival it is still difficult to achieve. The larval rearing of red porgy (*Pagrus pagrus)* and grouper (*Epinephelus marginatus*) is still difficult and with unpredictable results. The main problems, often reflected in massive mortalities, seems to be the identification of suitable feeding regimes for these species and to provide conditions of minimal stress. In order to tackle these problems, a large scale semi-intensive system (mesocosms) for rearing these species was developed and tested. Results were very promising for red porgy, wit the production of a large number of juveniles, but the system still needs to be tested for grouper.

Plan for 2005

The research during 2005 will continue to focus on various aspects of fish nutritional and digestive physiology, but also on broodstock management and aspects of larval and juvenile rearing of sole (*Solea senegalensis*), seabream (*Sparus aurata*), red porgy (*Pagrus pagrus)* and grouper (*Epinephelus marginatus*).

The effect of green water on larval development

Determine the influence of different microlage on:
a) fish growth through the analysis of RNA/DNA ratios, and protein turnover
b) lipogenic and amino acid metabolic enzymes in Senegalese sole
c) the nutritional quality of live food by biochemical analysis (portein and lipid content, fatty acids)

Understanding the regulation of the digestive function on marine fish larvae

Study the development of neuropeptides associated with the gastro-entero-pancreatic tract through imunohistochemical techniques, which in resemblance with other larval systems is poorly developed at first feeding although functionally adapted to this stage of development. According to existing bibliography, different periods can be identified on the neuro-endocrine system ontogeny, reflecting developmental differences in the regulation mechanisms of larval digestive function.

Physiological importance and metabolism of aromatic and sulphur AA during fish ontogeny

Preliminary work in order to obtain a better understanding on the utilisation of aromatic and sulphur amino acid along fish development will be carried out. Senegalese sole (Solea senegalensis), gilthead seabream (Sparus aurata), and toadfish (*Halobatrachus didactylus*) will be used as model species. This will allow comparing the amino acid metabolism in species with demersal and pelagic eggs and in species with marked metamorphosis.

Modelling the utilisation of dietary amino acids in fish larvae

The existing dynamic mechanistic model that simulates AA metabolism of fish larvae will be further refined. It will be used with different AA tracers, and it will be attempted to run it with more than one tracer amino acid simultaneously. The model will also be used to study the changes in AA metabolism during sole larval development. (Work in collaboration with Prof. Ivar Ronnestad, Univ. Bergen, Norway)

Effect of stressfull conditions on sole amino acid requirements

The relation between stressfull conditions and amino acid requirements on sole postlarvae and juveniles will be studied. Stress levels will be assessed through plasma/whole body cortisol,

glucose and lactate levels. Amino acid requirements will be studied studying plasma free amino acid levels, tracer studies, and intermediary metabolic enzyme activities.

Nutritional effects on fish proteome expression

Work will be continued on the effect of nutrition and feeding on zebrafish proteome expression. It is intended to do a preliminary study on the effect of dietary nitrogen type. Two-dimension gel electrophoresis will be followed by proteome comparision and identification of differentially expressed proteins.

Broodstock management

a) Identify the effect of using worms in the broodstock feeding on reproduction success and quality of eggs and larvae of sole.

b) Reinforce, in collaboration with fisherman, the existing grouper and red porgy broodstocks. Adapt new fish to captivity.

c) Attempt hormonal induction of spawning in grouper using GnRHa slow-releasing devices and/or boosting injections. Induce precocious males using hormonal treatment.

Zootechnical aspects of larval and juvenile rearing

a) Determine the influence of dietary natural zooplankton, larval density and rearing volume in the growth performance, survival rates and fish quality for grouper and red porgy. Fish quality will be assessed based on morphological and biochemical criteria.

b) Determine whether a feeding hierarchy exist during weaning of Senegalese sole, how this eventual hierarchy is affected by diffeent zootechnical conditions, and how it relates with larval metabolism. This will be done using tracers in the food.

Division of Living Resources

Group: Marine Ecology and Evolution - MAREE

Research team

Leaders: Ester Serrão, Gareth Pearson

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Summary of activities and progress during 2004

Genetic Structure of Seagrass Populations

Main purpose:

Large–scale panmixis has long been the leading hypothesis for marine species, as marine habitats are often viewed as having few strong barriers to dispersal, possibly due to poor understanding of the nature of limitations to gene flow in marine environment. However, an increasing number of studies report high differentiation and restricted gene flow between marine populations, despite high dispersal potential. The elucidation of the factors responsible for such divergence is difficult but essential to understand evolutionary and speciation processes. In this topic we aim at inferring population genetic variability and differentiation at different spatial scales in order to provide insight in the predominant causes of genetic structure, allowing the identification of physical and biotic factors limiting gene flow. We use all four European seagrass species as model species for these questions because i) these are keystone ecosystem structuring species, ii) can have contrasting clonal and sexual colonization strategies and iii) there is widespread regression and loss of seagrass meadows and management predictions as well as restoration efforts should take into account genetic differentiation within and between populations and the factors inferred to influence these.

Achieved in 2004:

Continuation or completion of:

- Inferring genetic neighborhood size and dispersal distances in *Cymodocea nodosa* using spatial autocorrelation of microsatellite loci.
- Biogeography of *Cymodocea nodosa in the Canary Islands* inferred from microsatellite genetic markers.
- Phylogeography of *Posidonia oceanica*: Vicariance patterns in the Mediterranean Sea.
- Global phylogeographic analyses of *Zostera marina*, and *Zostera noltii*.
- Population genetic structure of *Zostera noltii* along W Iberia: consequences of small effective population size, habitat discontinuities, and current patterns.
- Methodological paper on combining power and efficiency in selecting genetic markers.

Plan for 2005:

Continuation or completion of:

- Review paper on methodological problems in the analyses of population genetic structure in clonal plants, and on new methods for describing genetic structure in clonal plants.
- Phylogeographic analysis of *Cymodocea nodosa* at the Atlantic-Mediterranean transition zone: sources and sinks, geographic isolation and current patterns.
- Spatial variation in patterns of clonal growth across meadows of *Posidonia oceanica* and its relationship with demographic status of the meadow.
- Mating system in *Posidonia oceanica*: Description of sexual reproduction: allo vs auto fertilization and respective survival of progeny issued from both.
- Do aquaculture cages have an effect on population genetic composition of adjacent seagrass meadows versus non-adjacent ones?

- How does population genetic structure of *Zostera noltii* differ from stable continuous meadows versus patchy meadows under frequent disturbance, in a permanent state of recolonization?

Population genetic structure of natural mangrove forests of Avicennia sp. in Vietnam: the recovery of genetic diversity 3 decades after Agent Orange

Main purpose:

Mangrove forests, together with seagrasses, are major keystone ecosystem structuring species along the coastlines of the world's tropical regions, and in addition to their ecological role their ecosystem services have high economic value, particularly as nurseries for commercially important species. However, there has been widespread mangrove reduction throughout SE Asian coastlines, associated with human activities. The aim of this research theme is the assessment of population genetic structure in disturbed mangrove forests in SE Asia. *Avicennia* spp were chosen as model species because these are not artificially planted and thus reflect ecosystem history.

The destruction of the Mekong Delta mangrove forests by Agent Orange is possibly the largest human disturbance event experienced by any ecosystem. The first objective of this research theme is the evaluation of the genetic composition of the mangrove forest recruited following the Vietnam war.

Achieved in 2004:

Continuation of:

- Mangrove Genetic Diversity Still Recovering 30 Years After Agent Orange: We described the genetic recovery of the mangrove *Avicennia alba* population in the Mekong Delta during three decades following the end of the war.
- Analysis of population genetic structure in *Avicennia marina* mangroves in Northern Vietnam and the Phillippines. Consequences of selfing and population size.

Plan for 2005:

- Genotyping more populations of *Avicennia marina* from Australasia (Central and Southern distribution range) for comparison with Vietnam (Northern distribution range).
- Submission of ms on recovery of genetic diversity of mangroves decimated by Agent Orange during the USA-Vietnam war.

Evolution of reproductive strategies in fucoid algae: Population genetic structure of dioecious versus self-compatible hermaphrodite species and their hybrids.

Main purpose:

The reproductive system shapes mating patterns thereby determining the organisation of genes in populations. Changes in the reproductive system can thus function as the drive for reproductive isolation and eventually for speciation. Population genetic studies can reveal the consequences of a particular reproductive strategy. In this theme we explore the relationship between reproductive strategies and population genetic structure in two closely related seaweed species, *Fucus spiralis* and *F. vesiculosus* that have broad areas of sympatry along their distribution. The opportunity to study alternative reproductive strategies in closely-related taxa is rare. The comparison of population genetic structure in *F. spiralis* and *F. vesiculosus* provides an excellent opportunity for the study of the evolution of reproductive strategies.

Achieved in 2004:

- Mating system: Five new microsatellite markers specific for *Fucus spiralis* were developed to complement the dataset acquired at five microsatellite loci previously developed by our team for *Fucus vesiculosus*. Using these markers, the implications of mating system for genetic diversity were assessed in the sister species *F. vesiculosus* and *F. spiralis*. Results revealed that *F. spiralis* reproduces mostly through self-fertilisation. The high selfing rates were associated with very low levels of genetic diversity but great interpopulation variations. These results were suggestive of a selective advantage of autogamy for increasing mating probability. Selfing in *F. spiralis* may have become advantageous by assuring its reproduction and thus increasing its capacity as founder of

new sites, and by enabling the specialisation of this species to its environment. However, the very low level of genetic diversity in this species may indicate a reduced ability to respond to environmental changes. Higher level of genetic diversity was observed in the outcrossing species *F. vesiculosus*. Some significant degrees of biparental inbreeding in this species were suggestive of short gamete dispersal or mating of spatially structured populations (ms in prep).

- Population genetic structure of dioecious versus hermaphrodite *Fucus* species: Genetic markers were used to assess the consequences of mating system and hybridisation for population genetic structure of *F. spiralis* and *F. vesiculosus* in sympatric and allopatric zones along their distribution. Complete reproductive isolation between the two species was observed in allopatry whereas the occurrence of hybridisation and introgression was confirmed when the distributions of the two species overlap. Three main genetically distinct regions, possibly related to ice age refugia, present day marginal habitats and potential contact zone between differentiated populations, were defined. They were common to both species suggesting similar evolutionary history and barriers to gene flow independent of mating system. In contrast, mating system was important for genetic diversity and likely determined the contrasting distribution of the two species at their geographical limits. Finally, very strong genetic differentiation among *F. vesiculosus* populations indicates reproductive isolation within this species (ms in prep).

- Search for discriminant markers between *F. vesiculosus* and *F. spiralis* in order to resolve the direction of hybridisation. One microsatellite marker developed using *F. spiralis* genomic DNA did not cross-amplify in *F. vesiculosus*. Amplification and sequencing of this marker in both species using two different pairs of primers suggested the absence of this locus in *F. vesiculosus* genome.

Plan for 2005:

- The pattern of genetic differentiation between species, among regions will be assessed to provide clues as to the maintenance and evolution of the different reproductive strategies of the two species.

- Search for cytoplasmic discriminant markers between *F. vesiculosus* and *F. spiralis* in order to resolve the direction of hybridization. Sexual competition experiments between these species and their hybrids.

- Mechanisms of reproductive isolation between the two species: The influence of mating system and local adaptation/selection on gene flow between the two species will be assessed using a combined ecological (laboratory and field crossing) and population genetic approach.

- Population genetic study of *F. vesiculosus* in the Ria Formosa, Portugal: The objectives will be to determine (a) where *F. vesiculosus* populations from the Ria Formosa come from, (b) if *F. vesiculosus* reproduce sexually in the non rocky environment of the Ria, what is the potential for clonality in this environment and (c) if *F. vesiculosus* from the Ria is locally adapted.

STRESSREG The molecular basis for differential stress-tolerance in coexisting, ecologically similar algal species.

Main purpose:

Populations isolated near their limits of distribution experience strong selection pressure from abiotic stress and undergo accelerated, environmental stress-driven evolution. Such populations may thus become genetically differentiated, as a consequence of both limited interpopulational gene flow due to geography, and local adaptation to their environment. In order to test these predictions, the project addresses two questions 1) has stress-driven evolution occurred in populations along an environmental stress gradient (local adaptation)? 2) What are the consequences of local adaptation to stressful environments on population structure? Local adaptation may indeed change genetic characteristics of a population, such as mating system, or dispersal, which are reflected in population structure. We aim at combining information from selective traits and neutral genetic markers to study the evolution of local adaptation of edge populations of the intertidal alga *Fucus vesiculosus*.

Achieved in 2004:

- In order to identify partial genes involved in desiccation tolerance, and to identify genes that are differentially regulated at the population level (potentially involved in local adaptation) 6

subtracted cDNA libraries were constructed following desiccation and recovery experiments, for 3 populations of *F. vesiculosus* known to be physiologically divergent, by suppression subtractive hybridization (SSH) (2 libraries for up-regulated genes and 2 for down-regulated genes). A full-length cDNA library was constructed for *F vesiculosus* that contains up-regulated genes for desiccation and recovery processes. Subtracted libraries were screened to identify differentially expressed genes. After differential screening, approximately 2000 clones were sequenced from desiccation libraries, and 1000 from recovery libraries. These EST collections have been assembled into contigs and submitted to database searches (Blastx and Blastn). Most contigs have no homology to sequences in the databases, but several have homology to stress-related genes (LEA-like proteins, metallothionein), and genes involved in cell wall modification (extensins, *Chlamydomonas* zygote-wall specific protein). There is considerable evidence for polymorphism at the population level, as well as possible alternative transcripts. Macroarrays have been designed for unique sequences from both desiccation and (ongoing) recovery libraries. Experimental RNA samples for desiccation and recovery from several populations (central Atlantic, Portuguese western and southern Atlantic coasts, Baltic Sea and North Sea) have been prepared and will be used to probe macroarrays to investigate population-specific gene expression.

- Using microsatellite markers, we have identified strong differentiation within the species *Fucus vesiculosus*, where populations were shown to cluster not only according to geographic separation of central versus southern limit locations, but also according to habitat characteristics. There is evidence for either sympatric ecotypic differentiation or multiple colonizations by differentiated sources, and these are still maintained as distinct despite being sympatric.

Microsatellite data have also shown that genetic diversity decreases from the center towards the species margin, whereas the genetic variation between populations increases in populations at the limit, as would be expected from the balance between selection, migration and drift within marginal populations as compared to central populations (ms in prep).

Plan for 2005:

- Positive clones obtained from screening cDNA libraries will be to probe full-length cDNA libraries to full gene sequences and to identify stress-responsive genes using bioinformatic approaches.
- Macroarray analysis of stress-responsive gene targets to assess variation in gene expression at the population level, and to identify genes potentially involved in local adaptation.
- Crosses between populations that are highly differentiated in order to evaluate their cross-fertility and their fitness relative to within-population crosses (testing for inbreeding/outbreeding depression).
- Microsatellite analysis of *Fucus ceranoides*, a sister species to *F. vesiculosus* but that is restricted to estuarine habitats, in order to evaluate the taxonomic position of the estuarine types relative to this species.

Circadian and circatidal cycles and signals for the chloroplast: photosynthetic physiology and gene expression responses in *Fucus*

Main Purpose:

Recent investigations of gene expression by our team, have shown that chloroplast-encoded ribulose-1,5-bisphosphate carboxylase (rbcL, rbcS), ATP synthase (atpH, atpI), and photosystem I chl a binding protein (psaA) mRNA abundances vary in response to irradiance and immersion/emersion in *Fucus vesiculosus*. We now aim to evaluate whether the variability in photosynthetic parameters and gene expression in *Fucus vesiculosus*, detected in our previous studies, is related to circadian and/or circatidal signals and/or endogenous rhythms. The work involves determining gene sequences from macroalgal chloroplasts (plastids) to study their regulation and control of expression in photosynthetic organisms living in intertidal environments. Patterns of chloroplast-encoded gene transcription and adundance in intertidal fucoid algae in relation to circadian and tidal rhythms will be revealed for the first time. Environmental and/or endogenous cues with a role in regulating chloroplast mRNA abundance will be identified

Achieved in 2004:

1. Sequencing of > 90% of the chloroplast genome in *Fucus vesiculosus*
2. Completion of chloroplast genome map (140 kbp).
3. Completion of probe amplications for gene arrays. Optimization of cDNA labeling techniques.
4. Ecophysiological experiments to determine whether the chloroplasts have circadian and/or circatidal rhythms.

Plan for 2005:

- RUBISCO gene expression and temperature stress experiments.
- Circadian rhythms and light quality experiments (physiological).
- First application of chloroplast gene arrays (temperature/light experiments).

Ecophysiology of gamete release by intertidal species with external fertilization

Main purpose:

Fucoid algae, like many marine organisms, rely exclusively on external fertilization in the water column for successful reproduction. We use these as model species to investigate how intertidal species with external fertilization can achieve high reproductive success despite the gamete diluting effects of high water motion and whether external fertilization takes place at either slack high tide or as soon as organisms are reached by the incoming tide, as these are the phases that minimize gamete dilution. It is also asked whether for these intertidal populations gamete dispersal is restricted and whether the bottleneck between the large amounts of gametes that are released and the few juveniles that are found is not fertilization success but is instead early post- settlement survival to recruitment.

Achieved in 2004:

Completion of:

- Annual, monthly and daily periodicity of gamete release has been estimated in *Fucus vesiculosus* and *F. spiralis*, by sampling gamete release and egg settlement.
- Estimation of reproductive success of these two Fucus species with contrasting reproductive systems, assessed as fertilization success, recruitment and recruit survival.
- Publication of our results showing the spectral light signals for synchronous gamete release in *Silvetia compressa*. The spectral characteristics of light-induced gamete release suggest that the blue-green light environment in many coastal waters may induce gamete release from receptacles at high tide. The proximity of the effective wavelengths for the opposing processes of light-induced gamete release (effective in blue and blue-green to at least 515 nm) and de-potentiation (maximum measured effectiveness at 530 nm) suggest that the ability of receptacles to de-potentiate in green light reflects the relationship between turbidity (and therefore often in turbulence) and a shift in light transmission in the water column towards longer wavelengths, functioning as a second sensing mechanism for water motion, in addition to the sensitivity to inorganic carbon supply detected in our previous studies.

Plan for 2005:

- We will initiate population genetic approaches via spatial autocorrelation analysis of microsatellite genetic data to compare the dispersal of gametes and zygotes within populations between the hermaphroditic and dioecious species.
- Estimation of gamete release and reproductive success patterns in Fucus species along sympatric distribution zones in Brittany, France.
- Preparation of ms on reproductive ecology of Fucus species with contrasting mating systems.

Colonization strategies and population genetic structure of invasive algae: the genus Caulerpa in the Mediterranean Sea

Main purpose:

Caulerpa spp are clonal marine algae, which have been shown to act as invasive species, and reported to outcompete seagrasses. In the Mediterranean, two exotic *Caulerpa* species, *Caulerpa taxifolia* (probable introduction via aquaria from Australian sources) and *Caulerpa racemosa* (migrant from the Red Sea) have spread into areas formally occupied by seagrasses, and there is concern about the potential environmental and economic negative effects on the ecosystem. There is also a native species, *Caulerpa prolifera*, which opportunistically occupies disturbed *Posidonia* meadows. Very little is known about the reproductive strategies, clonal diversity and population structure of these species, despite growing concern about their spread as invasive species. The purpose of this project is to examine patterns of clonal growth, clonal structure and genetic composition of recently established populations of *Caulerpa taxifolia* and *Caulerpa racemosa* with populations of the native *Caulerpa prolifera* in the Mediterranean.

Achieved in 2004:
Development of microsatellite markers for *Caulerpa prolifera*. Field studies to estimate the spread of this species by asexual or clonal reproduction. Sampling of populations for genotyping.

Plan for 2005:
Completion of the development of microsatellite markers for *Caulerpa* species. Continuation of field studies and sampling.

Group: ALGAE - Marine Plant Ecology

Research team

Leader: Rui O. P. Santos
Post-docs: Raquel Carmona, Alexandra Cunha, Aschwin Engelen, João Silva
PhD students: João Silva, Raquel Machás, Susana Cabaço, Cecile Godinho, Leonardo Mata, Andreas Schuenhoff, Estibaliz Berecibar
Master's students: Ana Alexandre, Vasco Vieira, Ana Rosa
Research Assistants:, Helena Barracosa
Technicians: Catarina Alves, Luís Dias, Carla Domingos, Rui Candeias, Paula Seixas, Dora Rio
Honours Students: Ana Barradas, Rodrigo Delgado, Vânia Raposo

Secretariat: Ana Cruz
Diving officer: Pedro Neves

Summary of activities and progress during 2004

1. Metabolism of coastal systems
The research on the metabolism of coastal systems such as Ria Formosa lagoon aims the role of biota, particularly of plants, in the organic matter, nutrients and gas (e.g. O_2 and CO_2) fluxes in the ecosystem. The understanding of ecosystem function will allow addressing important processes, such as the human utilization of the ecosystem, the regulation of atmospheric CO_2 concentrations, or global change scenarios.
The specific lines under research in 2004 were:

Seagrass nitrogen uptake
The seagrass species *Zostera noltii* is a key species of the Ria Formosa ecosystem, playing a crucial role on the cycling of the nitrogen load of the system. The mechanism through which the nitrate is incorporated in the plant is catalyzed by the enzyme nitrate reductase (NR), whose activity is related to the nitrate uptake efficiency. The activity of this enzyme in seagrasses is poorly known despite the importance of seagrasses in the nitrogen cycle of coastal systems. NR activity is being investigated in our laboratory using two methods, an in vivo method, without cell disruption and an in vitro method where the enzyme is extracted from the cell tissue. Alexandre et al. (2004)

revealed that the NR activity of *Z. noltii* varied along the intertidal distribution of this species in Ria Formosa lagoon and that temperature but not pH had a significant effect on the enzyme activity. Further investigation on the effects of the environmental factors on NR activity will be developed to understand the determinants of the nitrate uptake by this species.

Photosynthetic ecophysiology of seagrasses

To understand the underlying mechanisms that determine the production of *Zostera noltii* in Ria Formosa lagoon, the photosynthetic ecophysiology of this species is being investigated. The use of chlorophyll *a* fluorescence as a valid proxy for photosynthetic production was validated for *Zostera noltii* by Silva and Santos (2004). This is a crucial step that allows the use of rapid, *in situ*, assessment of this species photosynthesis. The seasonal photosynthetic strategy of the seagrass *Zostera noltii* in Ria Formosa lagoon was also assessed. A manuscript presenting this data is under revision.

Metabolism and carbon fluxes of western Ria Formosa

The metabolism and carbon flux in the western sector of the Ria Formosa coastal lagoon were assessed to elucidate the relative importance of the contribution of the main communities, the treated sewage inputs from the adjacent city of Faro, and the exchange with the adjacent coastal waters to the ecosystem metabolism (Santos et al. 2004). The results depict the Ria Formosa as being a highly productive ecosystem dominated by the seagrass *Zostera noltii.* The community dominated by the seagrass *Cymodocea nodosa* had half of the gross production of *Z. noltii,* followed by bare sediments and phytoplankton. The net contribution of seagrasses to community metabolism was negligible, as both *Z. noltii* and *C. nodosa* showed a production: respiration ratio close to 1. Benthic microalgae emerge as the most important components of the net metabolism. The western sector of Ria Formosa was in metabolic balance during the summer when the study was done. The Ria Formosa ecosystem is shallow and rapidly flushed by the tides, which force an important exchange of dissolved organic carbon (DOC) and particulate organic carbon (POC) with the adjacent coastal waters. The daily net export rate to the adjacent coastal waters, 0.98 Kmol d21, represented 7.6% of the net ecosystem production, suggesting that the bulk of the net ecosystem production accumulates within the ecosystem. The western sector of Ria Formosa has an organic carbon sink of about 46.4 tons per year. Most of this is harvested in the form of molluscs (clams, cuttlefish, etc.) and fish (sea bream, sea bass, etc.).

The carbon and nitrogen fluxes of biomass of the seagrasses and saltmarsh plants to the food web of coastal systems are mainly done through the leaching of dissolved organic matter and through a long decomposition pathway of more resilient biomass. We have investigated how the isotopic composition of *Zostera noltii* leaves varied during the early phases of decomposition. This is important information to use when interpreting the food web relationships using the stable isotope composition of food web components. A manuscript was recently accepted for publication presenting these results.

2. Human impacts on coastal Systems

This research line focuses on the effects of the human-related disturbances on the seagrasses and salt marshes of the most important costal systems of southern Portugal. Our aim is to contribute with sound scientific inputs to the conservation and management of these systems.

A major threat to the coastal ecosystems results from the increasing nutrient load with antrophogenic origin. The results of the research developed in the last years on the characterization of the urban effluents from a waste water treatment plant of Faro, Ria Formosa lagoon, on the biological uptake by the communities and on the impact on the populations of the seagrass *Zostera noltii* were analyzed and included in four manuscripts. Three are being prepared while a fourth was already submitted and accepted for publication.

Another major threat of seagrasses is the physical disturbances caused by resource exploitation, such as clam harvest. In the Ria Formosa lagoon the clam harvest activity is the main commercial activity in this system. The population and reproductive level effects of clam harvesting on the seagrass *Zostera noltii* were investigated. Two manuscripts are accepted for publication presenting the achievements of this research.

A natural physical disturbance of seagrass meadows of Ria Formosa lagoon is related to the natural patterns of inlet migration. The impacts of the migration of the Ancão inlet on the *Z. noltii* meadows were assessed during the last years. The results were analysed and a manuscript was prepared and submitted. It was recently accepted for publication.
Dredging can be another main cause of seagrass decline. An assessment of the seagrass distribution and cover of the Arade estuary, Portimão, was performed to define the baseline seagrass condition to monitor the impacts of dredging a navigation channel from the estuary to the upriver town of Silves. This was done under the project "Caracterização dos bancos de fanerogâmicas e macroalgas no estuário do Rio Arade", Contract Instituto Portuário do Sul/CCMAR.
During 2004 our group contributed to several chapters of a book aiming to give environmental managers a basic introduction to monitoring and management of European seagrasses, "European seagrasses: an introduction to monitoring and management" (eds)J Borum, C M Duarte, D Krause-Jensen and T M Greve, EU project Monitoring and Managing of European Seagrasses (M&MS), EVK3-CT-2000-00044, http://www.seagrasses.org:
- Cunha, A. H., Duarte, C M., and Krause-Jensen, D. How long it takes to recolonize seagrasses?
- Krause-Jensen, D., Almela, E. D., Cunha, A. H. and Greve, T. M. Have seagrass distribution and abundance changed?
- Krause-Jensen, D., Quaresma, A. L., Cunha, A. H. and Greve, T. M. How are seagrass distribution and abundance monitored?
- Borum J, Greve T M, Binzer T and Santos R. What can be done to prevent seagrass loss?
- Duarte C M, Marbà N and Santos R. What may cause loss of seagrasses?
- Marbà N., Duarte C.M., Alexandre A., Cabaço S. How do seagrasses grow and spread?
Another contribution to give environmental managers a sound scientific background was done in Santos (2004), on the marine benthic flora, in *Encyclopedia of Life Support Systems* (EOLSS), developed under the auspices of the UNESCO.

3. Economic valorization of seaweeds
The research on the economic valorisation of seaweeds focus on the red alga *Gelidium sesquipedale*, which is harvested in Portugal for the extraction of agar and on the integrated aquaculture of seaweeds in fish farms, in order to biofiltrate their effluents and to produce economically valuable biomass. A manuscript was submitted on the research developed on the ecophysiological performances of diploid, male and female fronds of the red alga *Gelidium sesquipedale* to assess whether the unequal distribution of the two life cycle phases is related to a better fitness of diploids. As well, a modelling approach to the vital rates determinants of the haploid/diploid population ratio was developed. A manuscript is being prepared with this subject.
On what concerns the use of seaweeds to biofiltrate the fish farm effluents, three manuscripts are accepted for publication reporting the achievements of our group on the EU project SEAPURA, Species diversification and improvement of aquatic production in seaweeds purifying effluents from integrated fish farms. A Special Issue of the journal Aquaculture was edited by Santos, R., including the whole Seapura project achievements and some invited communications on integrated aquaculture. This Special Issue is in press.

4. Effects of global change on the Portuguese marine flora
The Portuguese continental coast has been recognized as particularly relevant in biogeographical terms (Lusitania Province), as it represents a boundary between the southern warmer communities and the northern colder communities, and hence has a high biodiversity. It has been reported that more than 40 northern species and more than 20 southern species have their distribution limits in Portugal. We are assessing the long-term changes in the benthic marine flora by comparing the actual situation with the only available description of the Portuguese marine flora, which was done in the 1960´s by Ardré. The relationships of the patterns of change, particularly the changes in the distribution limits and the origin of the new introduced species, with the water temperature increase will be assessed.
The global development of marine communications through boating and the wide geographical transplant of aquaculture species have increased the number of introduced species. Some of this

can eventually become invaders. The brown seaweed *Sargassum muticum* originates from Japan. It has invaded the American west coast from Alaska to Mexico in about 50 years and the European coasts from Norway to Portugal in about 30 years. In sheltered habitats, the species develops extensive masses, dominating the system and being a nuisance to local users such as tourists and fishermen. The population biology of *Sargassum muticum* and the competition dynamics with local species are being investigated in its southern distribution limits, in southern Portugal. A manuscript is being prepared on the periodicity of propagule expulsion and settlement in the competing native brown seaweed *Cystoseira humilis* and the invader *Sargassum muticum*, to be submitted in 2005.
In order to assess the relationships among the edge populations of the *S. muticum* geographical distribution with the source populations of propagules that determine the invasion potential of the species, we started developing molecular markers for this species, namely multisatelites. This research line will be developed in subsequent years.

5. Environmental Education

The Environmental Education Centres (EEC) correspond to all the initiatives, including appropriate installations and specialised educational teams, which offer environmental education programmes and activities. The research developed aims to evaluate how established are the EECs in Portugal as well as the limitations and problems encountered in their educational action. This line of research is unique in our country where the environmental education area is still little developed.
The environmental education develops from the realization of the environmental crisis that we face and from the necessity of an urgent social intervention to contribute to its solution. Considering that the educative interventions are slow and progressive processes that don't produce immediate changes on people, it is not easy to evidence a direct relationship between the improvement of some environmental conditions or the decrease of certain ecological problems and the specific educative intervention. The approach of Significant Life Experiences (SLE) may contribute to this problem's resolution. The analysis of the life experiences permit to detect the formative influences, which, in the subjects' opinion, were determinant in the development of environmental awareness.
Achievements:

1. The Portuguese EECs were quantified and described in detail according to selected dimensions such as the infrastructures and installations, the human resources and the educational programmes offered.
2. An evident contrast was observed between the increasing number of EECs in Portugal,in recent years, and the problems revealed in many centres, that is to say, the quality and the quantity did not have the same dynamics of growth.
3. A set of criteria has been defined to synthesise and quantify the present situation of the EECs in Portugal. They will be the pilot document to develop into the "Quality standards for the EECs in Portugal".
4. The preliminary results of the SLEs indicate that "Childhood nature experiences" and "EECs activities" are the most referred single factors in the development of environmental awareness.
5. We organized the first Portuguese meeting on "Environmental Education Centers: realities and perspectives", held at the Environmental Institute, Lisbon, November 19, 2004.

Plan for 2005

We plan to develop further the following research lines, besides continuing those financed by ongoing projects.

1. Metabolism of coastal lagoons

This research line will be continued to extend the acquired knowledge to other biological communities of Ria Formosa lagoon and to assess the seasonal variation in the metabolic processes of community production and respiration. A special methodology was developed for the first time, to be able to assess the community production and respiration when submerged, measuring CO_2 fluxes (João Silva, Rui Santos, Maria L. Calleja, Carlos M. Duarte 2005. Submerged versus air-exposed intertidal macrophyte productivity: from physiological to community-level assessments, Journal of Experimental

Marine Biology and Ecology 317: 87– 95). This methodology will be further developed for technical optimization and will be extensively used to collect field data. Two projects were submitted to national funds to develop this research.
Further investigation on the effects of the environmental factors on NR activity will be developed to understand the determinants of the nitrate uptake by this species. To attain this it will be necessary to solve the methodological problems related with the methods used, particularly with the *in vitro* method, which is not optimized for seagrasses. This was already initiated and will continue until a final protocol is operational.

2. Human impacts on coastal systems
We plan to assess an important environmental problem of the Ria Formosa lagoon and adjacent coastal zone, related to the development of green algal blooms. This phenomenon is related with the increasing load of dissolved inorganic nitrogen of the water and has caused major ecological damages in many coastal zones of the world with important economical impacts, particularly on the tourism industry. A post doc was recently hired o develop this research line and a project was submitted to national funding. A Marie Curie European Re-Integration grant was awarded to finance the first year of the post doc research.
A research line related to the importance of the seagrass meadows to the recruitment of the bivalve species in Ria Formosa lagoon is also being initiated. Preliminary field and laboratory work is being developed and a project was submitted for national funding.

3. Economic valorization of seaweeds
Two Ph D grants were awarded to members of the group to continue the research on integrated aquaculture using seaweeds of the Order Bonnemaisoniales, which was subject of a patent submission (Logogne V, Patrick D, Lunning K, Santos R, Mata L, Bansemir A, Schuenhoff A and Lindequivist U, 2003, procedé de production a terre des algues rouges de la famille des Bonnemaisoniacées. Submission nº 0308717, Institut National de la Proprieté Industrielle, Paris, França). Both the cultivation system processes and the ecophysiological determinants of the metabolism of the halogenated compounds responsible for the biological activity against pathogens will be further investigated. As well, cooperation with Zoomarine, a marine theme park of Algarve, was developed and a project was submitted for national funding to install a seaweed-based biofiltration system at Zoomarine.

4. Effects of global change on the Portuguese marine flora
To develop further the research line of the invasion of southern Portugal by the brown seaweed *Sargassum muticum*, we will test if the obtained microsatelites are polymorphic and thus if they can be used to assess the population genetic ecology of the edge populations of this species. As well, we plan to assess the biogeographical patterns of invasion, analysing samples from the original geographical distribution and from the introduction sites in Europe. A project was submitted for national funding with this subject.

5. Environmental Education
We plan to develop further this research line by implementing a set of discussion groups throughout the country, including representatives of Environmental Education Centres (EEC), visitors and sponsors to validate the criteria that were defined to quantify the quality standards for the EECs in Portugal. As well, the state of the art of the research on environmental education in Portugal will be assessed based on the content analysis of the published thesis and dissertation on the subject.

Group: Ecohidrologia e Recursos de Estuários e Zonas Costeiras (Ecorecursos)

Research team

Leader: Luis Chícharo, Alexandra Chícharo
Visiting scientist: Eric Wolanski

Post-docs: Marina Delgado, Radhouane Ben Hamadou
PhD students: Pedro Morais, Joaquim Teodósio, Ana Amaral, Rita Borges, Vanessa Moschino, Adriana Candeias
Honours thesis students: Ana Faria, Joana Dias
Research Assistant: Isabel Marques

Summary of activities and progress during 2004

Main purpose:
1- To study the impact in river inflow in estuarine and coastal biodiversity. Within this aim, the group collaborated with UNESCO IHP (International Hydrologic Programme) under the Ecohydroloy programmne
An ecohydrology model has been developed and applied to the low flow conditions in the Guadiana Estuary in collaboration with the visiting scientist Dr. Eric Wolskanki. The model integrates physical and biological processes in the estuary and it predicts the ecosystem health as determined by the following variables: nutrients, suspended particulate matter, phytoplankton, zooplankton, bivalves, zooplanktivorous fish and carnivorous/omnivorous fish. The model is verified against field observations of these variables. It is shown how the model can be used to quantify the impact on the health of the estuary of flow regulation through dams, particularly the giant Alqueva dam, and of changes to land-use through irrigation farming, and the efficiency or otherwise of various ecohydrologic remediation measures. The model is a tool that may enable an interaction between scientists, economists, the public and decision makers to enable sustainable development of the Guadiana River catchment based on ecohydrology principles.
-Participation in UNESCO Scientific Advisory council on Ecohydrology as a tool for restoration and management of the coastal zone, meeting in Paris May 2004.
-Organization of Workshop "Managing the Guadiana estuary – the Ecohydrology and Phytotechnology approaches " under the framework of the IHP-UNESCO

Plans for 2005-2006

To continue research lines started, with particulary emphasis in:

- Remediation and mitigation studies of the impacts of anthropogenic activities on the ecosystems, with emphasis in the Guadiana demo Site
- Ecophysiological and biochemical studies of exotic versus indigenous species
- Condition and retention mechanisms of estuarine and coastal fish larval
- Organising an international Congress on Ecohydrology, under the support of UNESCO.

Group: Crustacean Biology and Fisheries

Research team

Researchers: Margarida Castro, Margarida Cristo and Margarida Machado.
PhD students: Paula Serafim.

Summary of activities and progress during 2004

Non-cladoceran branchipods in temporary ponds
Main Purpose:
Obtain an inventory of species present in ponds form the South of Portugal. To study of the biology ans population dynamics of the most important species:
Achieved in 2004:
1. Identification of species present in 19 ponds.
2. Registration of 1 new species for Portugal.
3. Registration of one new species worldwide.

4. Following of the biological cycles of 4 species in 7 ponds.
5. Study of the accompanying fauna, mainly amphibians.

Survival of invertebrate non-target species of crustacean trawling, discarded on board

Main Purpose:

To obtain estimates of survival rates of discarded invertebrates. To assess stress and damage cause by capture and release.

Achieved in 2004:

1. Laboratory experiments to study physiology of controls.
2. Data analysis

Spiny lobster biology and management

Main purpose:

To update the information of the biological cycle of *Panulirus elephas*.
Characterization of the fishing activity of the artisanal fleet in the port of Sagres.
Set up of lobster larvae collectors for estimation of recruitment.

Achieved in 2004:

1. Study of impact of gillnets in accompanying species.
2. Study of the biology of the spiny lobster bases on onboard sampling.
3. Choice of locations to setup larval collectors

Plan for 2005

- Continuation of the ecological studies in temporary ponds. A sample of the previously studied ponds will be observed for understanding year-to-year variation. Submission of the newly recorded species to international zoological boards.
- Continuation of the feeding ecology of deep water decapods
- Seting of *puerulus* collectors for estimation of temporal and spacial paterns of settlement for the rock lobster *(Panulirus elephas)*

Preliminary studies on the viability of spinilobster aquaculture, based on the collection of wild *Puerulus*.

Group: Fisheries Biology and Hydrobiology

Research team

Leader: José Pedro Andrade
Principal researchers and post docs: Pedro Domingues, Jorge Palma
PhD students: Eduardo Esteves, António Sykes
MSc student: Miguel Correia

Summary of activities and progress during 2004

Cuttlefish culture:
During 2004, research was focused on the use of nucleic acid derived indices and instantaneous growth rate as tools to determine different nutritional condition in cuttlefish hatchlings
Groups of cuttlefish hatchlings will be used to determine the duration of the yolk reserves, during which growth can be obtained with no food supply. These were fed live grass shrimp ad libitum from the third day of life onwards Nucleic acid derived indices and instantaneous growth rates (IGR) were used as a way to describe their condition, when all the yolk reserves were exhausted and to determine to most accurate tool to express growth and condition.

Plan for 2005

Cuttlefish culture

During 2005, the effects of culture density on growth and broodstock management of the cuttlefish will be studied.
The primary objective of this research will be to study the effects of a high and a low culture density in juvenile growth, feeding and food conversion; and secondly, to access the effects of mortality and crowding on growth and reproduction (fertility and fecundity) of cuttlefish. The secondary objective will be used to determine a good culture density that will enable the establishment of broodstock management guidelines. The effects of different coloured tanks on growth and reproductive performance will also be studied. The objectives of this experiment will be to access the effects of three different colours on hatchlings growth, feeding rate, food conversion and survival; on juvenile growth and on adult reproductive performance.

Crustacean research
Throughout 2005, the crustacean research will focus on larval development and production enhancement of the species *Palaemonetes varians*, *Palaemon elegans*, *Necora puber* and *Pagurus pridauxi*. Biological research will focus on the study of the larval stages until settlement, larval mortality and optimal growing density. Mass production studies will focus on growth rates enhancement with different diets and optimal rearing systems.

Group: Coastal Fisheries Research

Research Team

Leader: Karim Erzini
Post Doc Researcher: Jorge M.S. Gonçalves
Researchers: Luis Bentes, Joaquim Ribeiro, Pedro Monteiro, Rui Coelho, Carlos Afonso, Humberto Hazim, Daniel Machado, Pedro Veiga, Frederico Oliveira and Cheila Almeida.

Summary of activities and progress during 2004

Ongoing Projects:

1. - “Experimental By-catch Reducing Devices (BRD) in the demersal purse-seine fishery and evaluation of survivorship”. (Project Ref.: FCT - POCTI/BSE/43113/2001)
2. -“Avaliação experimental do impacte nas biocenoses marinhas associado à exploração de manchas de empréstimo ao largo do Algarve Central” (DRAOTA-RENSUB).
3. -“Recrutamento de Espécies Piscícolas de Interesse Comercial no Estuário do Rio Arade” (DGPA – MARE P.O. Pescas: 22-05-01-FDR-00017).
4. - "Scientific bases for the management of fisheries resources of common interest" (GESTPESCA-INTERREG III Project).

Plan for 2005

The group will focus on the biological impact assessment of coastal dredging; the seabed mapping and sensibility/vulnerability analysis; recruitment of commercially important species in the Arade estuary (Algarve) and the monitoring of the ichthyofauna of the Ria Formosa. Studies on fisheries biology and population dynamics of swordfish and deep water sharks will continue. Another important line of research will be habitat use and spatio-temporal distribution of fish based on telemetry.

The evaluation of the impact of coastal dredging will involve sampling with a variety of gears and methodologies, including underwater transects by video and divers, beam trawling and bottom grabs and cores. Species will be identified, measured and counted in the laboratory. The data will be input into a GIS, allowing mapping of species distributions, composition and abundance as a function of bottom type and depth. This information, together with various indicators (e.g. diversity

indices) and the results of multivariate analysis will allow the identification of particularly important or sensitive areas. Special emphasis will be placed on the identification of essential fish habitats (EFH).

As part of the ongoing GESTPESCA I and II (INTERREG III) projects, the group will carry out telemetry studies in association with IPIMAR – CRIP-Sul. The objective is to gain a better understanding of the use of artificial reefs by reared and wild sea breams, especially the common sea bream (*Diplodus sargus*). To this end, pingers (Vemco Ltd.) are being implanted in reared and wild (caught at the reefs) common sea breams. The fish are released on the reefs and their daily movements recorded using hydrophones and receivers. A network of receivers around the reefs will record data on a long-term basis while a hydrophone on board a research vessel will be used periodically to study short-term movements. A newly approved FCT project will extend the telemetry work to the Ria Formosa lagoon where habitat use will be studied by monitoring various species of sea breams and sea bass.

Studies of the Ria Formosa fish community will continue with the annual monitoring program. A series of locations will be sampled with a beach seine in the Spring of 2005. This will continue the time series of sampling initiated in 2000 and considered of great importance for evaluating possible changes resulting from climatic or human-induced changes. The importance of lagoon and estuarine systems as nurseries and essential habitat for fish will also be studied in the Arade river. Here, a variety of sampling gears will be used to study the fish community along a salinity gradient and over tidal, daily and seasonal bases.

Another important line of research is that of population dynamics. Work will focus on the population dynamics of deep water sharks and swordfish in the south Atlantic. In both cases modelling will be used to evaluate the influence of fishing and environmental factors on these species.

Group: Biodiversity and Biology in Fisheries (BIOPESCAS)

Research team

Leader: Teresa Cerveira Borges
Post Doc: José Xavier
PhD students: Maria Esmeralda Costa, Sónia Olim, João Sendão
Technicians/ research assistants: Anxo Conde, Paulo Morais

Summary of activities and progress during 2004

1. Project "Science, Education and Marine Archaelogy Programme in Portugal" (SEMAPP)

During April 2004, a sea campaign took place in the man-submersible DELTA, from DELTA Oceanographics, USA. The DELTA submarine diving exploration of Portimão Submarine Canyon cruise for SEMAPP 2004 began on 5 April 2004 and terminated on 18 April 2004. In spite of limiting weather conditions (50% of the time) and periodic problems with ocean floor visibility and excessive background noise from ship propellers (N/R "Schultz Xavier"), affecting underwater communications, SEMAPP scientists, students and Portuguese Navy personnel worked together to accomplish the defined goals.

Summary of Dive Statistics

- Number of Dives Made 15
- Total Bottom Time 814 minutes / 13.6 hours
- Total Distance Transversed 8,670 m / 8.7 km /
- Total area of ocean floor surveyed 38,410 m^2
- Number of Portuguese Divers 8

- Number of American Divers 3
- Total Number of Participants (on Ship) 110
- Number of Agencies / Universities/ Foundations 15 (10 Portuguese, 5 American)
- Depth Range Covered Portimão Canyon, Nazaré Canyon 35-285 m

Entities Involved (funding / participants)

- Centre of Marine Sciences, University of Algarve
- Autonomous University (UAL)
- Faculty of Sciences, University of Lisbon (FCL)
- Portuguese Navy
- Portuguese Hidrographic Institute (IH)
- Ciência Viva
- Institute for Science and Technology (IST)
- Zoomarine
- Luso American Foundation (FLAD)
- Independent Communications Society (SIC)
- Ocean Technology Foundation (OTF)
- University of Connecticut (UCONN)
- National Undersea Research Center (NURC)
- Dreyfus Foundation
- R.A. Cooper Family

Accomplishments Science in Biology / Fisheries

Important new information on ocean floor biology / ecology and the impact of bottom fisheries on habitats was achieved on all of DELTA transects made. It should be noted that these direct observations and video documentations (approximately 30 hours) of ocean floor – faunal relationships and faunal behaviours, are the first ever recorded deeper than 100 meters in Portimão Submarine Canyon and adjacent continental shelf. Following are preliminary conclusions of biological and geological accomplishments.

- Observations (in-situ) and video documentation of ocean floor fauna and associated habitats in and around the head of Portimão Submarine Canyon, Algarve Coast over a depth range of 120 to 285 meters.
- Documentation of species behaviour and an estimate of species diversity and abundance.
- Populations structure and abundance of benthic / epibenthic fauna on fished and non-fished areas. Evidence of destructive fishing.
- Potential importance of discrete rocky habitats as “refugia” for ocean floor fishes, crustaceans and mollusks.
- Evidence of ancient shorelines, tsunamis or gravity falls at 190 to 220 meters in depth at the head of Portimão Canyon. Rock samples with encrusted oyster shells collected for carbon 14 dating.

Other Accomplishments

Ciência Viva

The national youth education program Ciência Viva was a strong participant in SEMAPP 2004. Two groups of students and teachers (high school) spent one day each on the ship; one student made a 30mn DELTA dive to 100 meters in Portimão Canyon. General reaction from both students and teachers – a thrilling, exciting and very educational experience. Reactions from Ciência Viva Administrators – a very worthwhile experience. Program should continue

Public Outreach

Several TV stations and newspapers cover the “yellow submarine” – Portimão Canyon event.

Zoomarine Turtle Release

Eight rehabilitated sea turtles (Loggerhead Turtles) were released at Portimão Canyon, successfully, in spite of rough seas. Excellent TV and press coverage occurred the following day.

2. Project "Cephalopod stocks in European waters: Review, Analysis, Assessment and Sustainable Management" (QOL-2001-5.1.2) (CEPHSTOCK)"

All existant biological data on cephalopods from previous research projects were analysed and several publications were prepared and submitted.

3. Project "Fishery of the common octopus in Algarve: Improvement of the Artisanal fishing gears (POLVARTE)" (Ref: 22-05-01-FEDER-00018)

During 2004, several controlled experiments were performed in CCMAR Marine Station of Ramalhete, to study octopus behaviour towards the new type of plastic pot, the traditional clay pot, the cylindrical plastic pot (already introduced in the Portuguese octopus fishery), as well as the iron trap "covo", also already used in the Portuguese octopus fishery and the only fishing gear using bate (specially sardine). The main questions were towards the preferences of octopus: what kind of material preferred – plastic or clay; what kind of shape – cylindrical or amphora shape; what colour – red, white or black; and is it possible for the octopus after tobe inside the iron trap "covo" to come out? The answers were that the common octopus prefers a pot with an amphora shape (more round in the middle), colour black and doesn't show any preferences towards the material. Concerning the behaviour towards the iron trap "covo", the common octopus comes in and out whenever he wants.

4. Project "Biodiversity in fisheries off the South coast of Portugal (Algarve) (BIOFISH)" (Ref: 22-05-01-FEDER-00031).

The proposal presented to Programme MARE, DGPA, at the beginning of 2004, was accepted. Start of project beginning of 2005.

Other studies

Post-doc José Xavier study projects (from Oct. 2004). The start of Post-doc at CCMAR focused on reviewing the importance of cephalopods in European waters, particularly South of Iberian Peninsula. The quality of datasets, collected in previous research projects, was also assessed and integrated into a framework of publishing papers. Further work related to the Antarctic was carried in two projects already initiated at British Antartic Survey (BAS), focusing on the predator-prey interactions in relation to environmental variables and fisheries. These two pos-doctoral research projects allowed the development of acoustic techniques, combined with trawling and biological sampling techniques, in order to understand the Antarctic fish fauna dynamics.

Plan for 2005

1. Project "Science, Education and Marine Archaelogy Programme in Portugal" (SEMAPP)

All video documentation will be analysed to estimate species diversity and relative abundance, as well as specific faunal behaviour. Analysis of trawl fisheries impact will also be documented and discussed. All results will be presented to the 40th European Marine Biological Symposium (EMBS) and published in the meeting proceedings or in a peer reviewed journal.

2. Project "Cephalopod stocks in European waters: Review, Analysis, Assessment and Sustainable Management" (QOL-2001-5.1.2) (CEPHSTOCK)"

This Concerted Action will finish at the end of 2005, with the presentation of a final report to EC and several scientific papers published in pieer reviewed journals.

3. Project "Fishery of the common octopus in Algarve: Improvement of the Artisanal fishing gears (POLVARTE)" (Ref: 22-05-01-FEDER-00018)

This research project will continue until middle of 2005 when it will finish, with the presentation of a final report to Programme MARE, DGPA. Experiments at sea, with fishermen, to compare octopus catches of different fishing gears (clay pots, cylindrical plastic pots, new plastic pots and

iron traps) will be conducted. It is expected to present several communications in different meetings and symposia, with the publication of several papers on octopus behaviour and comparison and effects of artisanal fishing gears. A meeting with fishermen and fishery managers will be conducted at 4 June 2005 to present results of this project and other research projects related to fisheries.

4. Project "Biodiversity in fisheries off the South coast of Portugal (Algarve) (BIOFISH)" (Ref: 22-05-01-FEDER-00031)

The main objective of this new project is the production of a book with all species caught (commercial and non-commercial) by the commercial fisheries, with different fishing gears (trawl, purse seine, trammel nets, etc). This book will present not only photos, but also biological, geographical and fisheries information of each species caught.

Other studies

Post-Doc study I (Portugal). Work will focus on a wide range of aspects of cephalopod ecology in European waters, including the South Coast of Portugal: 1) Characterisation of the cephalopod fisheries in South Portugal, 2) Evaluation of trends of cephalopod landings in Portugal and in Northeastern European waters, and 3) Preliminary assessment of cephalopod consumption by top predators.

Post-Doc study II (Antarctic). The research will focus on publishing data on the characterization of the vertical and horizontal distribution of the mackerel icefish *Champsocephalus gunnari*, and other Antarctic fish, in relation to the physical (temperature, currents, depth) and biological (e.g. Antarctic krill *Euphausia superba* distribution) characteristics in the Southern Ocean using a combination of trawling and acoustics.

Post-Doc study III (Antarctic). The research will focus on predator-prey interactions between top predators and cephalopods in Antarctic waters through the analysis of the diet of albatrosses, possibly king penguins, and collection of biological data of Antarctic squid.

Group: Biodiversity and Conservation (BioCon)

Research team

Leader: Rita Castilho
PhD students: Regina Cunha
Honors thesis students: Marta Freitas, Mafalda Barata

Summary of activities and progress during 2004

Molecular Evolution - Island Biogeography

To focus work on the Cape Verde archipelago as a model: to better understand animal colonization of islands and its evolution.

To organize the second "*Conus* Cape Verde Expedition II" to collect species of *Conus* (Gastropoda) and continue the work on molecular systematics and phylogeography of this marine gastropod genus which is the topic of a PhD thesis taking place at the Museo Nacional de Ciencias Naturales in Madrid, Spain, which I am co-supervising with Dr. R. Zardoya.

To access the suitability of other invertebrates and vertebrates species as models to infer the colonization and evolutionary history of the Cape Verde archipelago. This will involve field sampling as well as laboratory trials.

Phylogeny/Molecular systematics

To complete the data sequence analysis of the mtDNA 12S, 16S and cytochrome b gene fragments from Pleuronectiformes (Teleosts).

Phylogeography

To concentrate on the phylogeography of Portugal in order to test hypotheses on the processes responsible for shaping the genetic variation of species (e.g. habitat fragmentation, recurrent gene flow etc)

To complete the work on the molecular diversity and phylogeography of *Salamandra salamandra* (Amphibia) from Southern Portugal.

To collect blood samples of *Mauremys leprosa* (Amphibia) in order to approach the molecular diversity and phylogeography of the species in Portugal.

Achieved in 2004:

1. Publication/submission of papers from last year research activities on: 1) Population structure of anchovy (*Engraulis encrasicolus*) in the Mediterranean and the Atlantic: revealing strong unexpected subdivision(s); 2) Morphotype identification of stranded Azorean common dolphins, *Delphinus delphis*, by mitochondrial sequence genetic analysis; 3) Patterns of Cladogenesis in the Venomous Marine Gastropod Genus *Conus* from the Cape Verde Islands; 4) Population genetics of *Scomber scombrus* and *Scomber japonicus* in the Mediterranean and the adjacent Atlantic Ocean.
2. Completed a detailed sampling of *Salamandra salamandra* in Portugal, in view to increase sample size in previously under-sampled places and to sample new locations.
3. To start molecular work on the phylogeography of *Mauremys leprosa* through cytochrome *b* sequencing.
4. To work on the molecular systematics of sharks occurring in the Portuguese coast, from two different genus: *Etmopterus* and *Galeus*.

Plan for 2005

To continue the molecular phylogenetics and phylogeography approach initiated some 3 years ago:

1 - To finish the work on *Mauremys leprosa* and present a comparative approach as an oral communication at the "IV International Symposium on *Emys orbicularis*"

2 – To start a study on speciation processes of marine gastropods in Cape Verde, based on previous and new samples.

3 – To organize the "*II Molecular Evolution Workshop*" with the presence of David Swofford, Gavin Naylor and Mark Holder from Florida State University, USA.

Group: Fish Parasitology and Reproduction

Research team

Post-doc: Isabel Afonso-Dias (under the coordination of Manuel Afonso-Dias at Faculdade de Ciências do Mar e do Ambiente) with Ken Mackenzie acting as a consultant regarding Ichthyoparasitology.

Summary of activities and progress during 2004

The research of this group is mainly related to fisheries biology, particularly fish reproduction and the use of parasites as tags for stock identification. The studies carried out during 2004 included the following subjects:

1. "Gastrointestinal parasites of Sardine (*Sardina pilchardus, Walbaum 1792*): Study to assess the possibility of using sardines' parasites as biological tags"

This study started during February 2004 and is progressing according to plan. So far different species of digeneans (*Aphanurus* sp., *Hemiurus* spp.) and nematodes (*Anisakis* sp., *Hysterothylacium* sp.) were found in the stomach of sardine collected off France, Portugal and Spain. Although not all samples (viscera collection was made by different technicians / scientists) had gall bladder and/or liver, smears of these structures were made whenever possible to try to find myxosporeans and microsporideans. Hitherto two different species of myxosporean were found in the gall bladder of sardine. These species were sent to an Indian Colleague, Prof. Kalavati (Andhra University) for identification to species level.

2. "Anglerfish (*Lophius piscatorius and L. budegassa*) Parasites: Study to assess the possibility of using anglerfish parasites as biological tags"

A summary of the current status of this work was provided by Dr Ken Mackenzie and it was published (http://www.diplectanum.dsl.pipex.com/newsletter/2004/newsl11.htm#upd) in the International Ichthyoparasitology Newsletter, No. 11, January 2004, under the Current Research Activities in the United Kingdom.

Within the scope of this activity the paper entitled "A checklist of the protozoan and metazoan parasites reported from the anglerfishes *Lophius piscatorius* L. and *L. budegassa* Spinola" was published in the Bulletin of the European Association of Fish Pathologists (see list of publications).

Three different species of myxosporeans were found in the gall bladders of *Lophius piscatorius* and *L. budegassa* from west of Scotland and off the Algarve coast. When samples of these were sent for identification to Prof. C. Kalavati, they all proved to be new species. During 2004 a poster was prepared and presented in the IX EUROPEAN MULTICOLLOQUIUM OF PARASITOLOGY (EMOP IX), Valencia, Spain (Three new species of myxosporeans (Myxosporea: Bivalvulida) from the gall bladders of *Lophius* spp. (Pisces: Teleostei) in European waters), 18-23 July 2004. Detailed descriptions are now being prepared for publication.

Opportunistic samples of *L. piscatorius* from the West coast of Portugal were collected during 2004, whenever possible.

3. "Validation of the macroscopic maturity scale of Sardine (*Sardina pilchardus*, Walbaum 1792) currently in use"

This research activity consists of occasional consultancy and data analysis regarding routine histological examinations. During 2004, a manuscript entitled "Problems with assessing the ovaries of Sardine (*Sardina pilchardus*, WALBAUM) to the appropriate macroscopic maturity stage", was produced and will be submitted during 2005.

4. "Testicular infestation of *Sardine pilchardus* (Walb.) by the coccidia *Eimeria sardinae* (Thélohan)"

The activities in this field of research during 2004 included:

- Preparation and presentation of a poster in the IX EUROPEAN MULTICOLLOQUIUM OF PARASITOLOGY (EMOP IX), Valencia, Spain (Testicular infestation of sardine *Sardina pilchardus* (Walb.) by the coccidian parasite *Eimeria sardinae* (Thélohan, 1890)), 18-23 July 2004.
- Preparation and submission of a scientific project to FCT (Fundação para a Ciência e Tecnologia) in July, entitled Sardine (*Sardina pilchardus*, Walb.) castration due to the Coccidia Eimeria sardinae: exception or rule?" (POCTI/MAR/61732/2004).

5. "Comparing the parasite fauna of *Lophius* spp. with that of another top predatory fish, the halibut *Hippoglossus hippoglossus*"

This study that started in 2003, continued during 2004 with the participation of Isabel Afonso-Dias in a multidisciplinary research vessel survey along part of the coast of North Norway from 16 to 25 October 2004. The number of halibut sampled so far is still not sufficient to produce a coherent study on the parasitic fauna of these two top predators.

General Plan for 2005

1. "Gastrointestinal parasites of Sardine (*Sardina pilchardus, Walbaum 1792*): Study to assess the possibility of using sardines' parasites as biological tags"

This study will progress as planned to try to identify possible biological tags for stock identification. This study intends to complement the already in placement study of stock identification of this host species. During 2005 the laboratory work will progress as planned and it is also planned for the coming year to prepare written material for publication.

2. "Anglerfish (*Lophius piscatorius and L. budegassa*) Parasites: Study to assess the possibility of using anglerfish parasites as biological tags"

Like it was explained in the previous report, it is possible that the different species of the genus *Stephanostomum* could make useful biological tags. Since one of the main diagnostic features of these digeneans is the oral spines, that tend to disappear in frozen specimens, we are still examining fresh samples of *Lophius* spp., whenever possible. This is a very time consuming task and it will proceed, sporadically, during 2005.

After encountering three different species of myxosporeans in the gall bladders of *Lophius piscatorius* and *L. budegassa* from west of Scotland and off the Algarve coast, work will continue to:

a. Publish detailed descriptions of the species found;
b. To assess the possibility of using these species as biological tags;

3. "Validation of the macroscopic maturity scale of Sardine (*Sardina pilchardus*, Walbaum, 1792) currently in use"

This activity will be successfully finished during 2005, after the publication of the paper under preparation.

4. "Testicular infestation of *Sardina pilchardus* (Walb.) by the coccidia *Eimeria sardinae* (Thélohan)"

The activities planned in this field of research for 2005 are stationary until further notice from FCT. If the proposal presented to FCT is approved, then this activity will resume in 2005, if adequate.

5. "Comparing the parasite fauna of *Lophius* spp. with that of another top predatory fish, the halibut *Hippoglossus hippoglossus*"

During 2005 it is intended to carry on collecting more samples of *H. hippoglossus* and assess the viability of further developing this investigation.

Externally funded Projects

Division of Aquaculture and Biotechnology

New and ongoing beyond 2005

Title: Aquafirst- Combined genetic and functional genomic approaches for stress and disease resistance marker assisted selection in fish and shellfish
Summary and Objectives: The overall aim of this project is to identify genes associated with stress and disease resitance in oyster, trout, sea bream, and sea bass in order to provide a physiological and genetic basis for marker-assisted selection.
These studies will be carried out for fish using stress and pathogen challenges directly relevant to aquaculture and for oyster using environmental conditions that are known to lead to significant summer mortalities. In Part 1 of the project the genes involved in the functional response to stress or pathogen exposure will be identified. This will be carried out in the four species by (i) constructing relevant EST collections using SSH cDNA libraries which will be spotted on microarrays, followed by (ii) analysis of gene expression profiles in various tissues of animals exposed to stressors or pathogens. This analysis will be also carried out in families that are divergent for stress response or disease resistance (fish) or for summer survival (oyster). In order to investigate relationship between potential candidate genes and QTL for these traits, Part 2 of the project will identify Single Nucleotide Polymorphisms (SNP) in these candidate genes in oyster and trout. This polymorphism will be analysed both in the EST sequences and in the promoter region. In Part 3 of the project QTL analysis will be used to identify genes that are associated with stress specific traits and disease resistance traits using previously characterized SNP and also microsatellites markers. We will also carry out mapping of these genes in linkage and gene maps. Part 4 is devoted to outline operational genetic protocols incorporating identified QTL and traditional breeding approaches in oyster, sea bream and sea bass. This knowledge will be transferred to the industry through organisation of workshops gathering scientists and RTD performers.
Reference and funding entity: sixth framework programme - contract n° 513692
Duration: 1/11/2004 – 31/08/2005
Research team: Adelino Canário, Deborah Power, Pedro Guerreiro.
Total budget: 3,799,954 EUR Euro; **Funding for CCMAR**: 63 493 Euro
URL: http://lotus5.vitamib.com/hnb/aquafirst/aquafirst.nsf/Web/Frame?openform

Title: Marine Genomics Europe - Implementation of high-throughput genomic approaches to investigate the functionning of marine ecosystems and the biology of marine organisms

Summary and Objectives: The overall aim of this project is to set up and develop a European Network of Excellence, referred to as "Marine Genomics", for the implementation of high-throughput genomic approaches in the biology of marine organisms. "Marine Genomics" will promote, develop, and spread throughout the European Union a broad range of genomic approaches, to investigate a wide range of questions related to the functioning of marine ecosystems and to the biology of marine organisms. With this aim in view, we propose to group and network experts in genomics, proteomics, and bioinformatics from several Centres of Excellence in genomics in Europe with marine biologists who can make use of high-throughput genomics data. This will involve the dedication and the development of common research infrastructures, both in genomics and in marine biology. Joining together these distinct scientific communities will establish Europe's lead in marine genomics.

The J.E.R. of "Marine Genomics" is broken down into Comparative, Functional and Environmental Genomics, three sections which structure more traditional streamlines, leading to various microbial, algal, evolution development and diversity, and fish and shellfish nodes. This research can be applied to the management of marine ressources (prediction of global changes in marine populations, conservation of biodiversity, fisheries management and improvement of aquacultured species) and to gene mining for health and biotechnology.

The Integration activities of Marine Genomics are based on the following strategies: i) jointly develop enabling technologies; ii) sharing existing technical platforms iii) collectively gaining access to major Genomic centres; iv) regrouping under a common Bioinformatics Centre; and v), create and develop a Knowledge and Communication System, a permanent web-based interface for communications within and outside the network.

Spreading activities will include workshops and courses implemented by a Training & Education Council. Marine Genomics will also develop complementary dissemination strategies, targeting public, private and institutional communities with the purpose of enhancing the integration of marine biologists in the ERA.

Reference and funding entity: NoE sixth framework programme - contract n° 505403
Duration: 1/3/2004 – 28/02/2008
Research team: Adelino Canário, Leonor Cancela, Ester Serrão, Gareth Pearson, Deborah Power,Vincent Laizé, João Cardoso e outros.
Total budget: 10,000,000 EUR Euro; **Funding for CCMAR**: >100 000 euro
URL: http://www.marine-genomics-europe

Title: "Chemical Identification and Functional Roles of Reproductive Pheromones in the Tilapia, *Oreochromis mossambicus*"

Summary and Objectives: That pheromones play important roles in many aspects of fish biology is beyond doubt. However, the most complete evidence do date has been obtained from a few well-studied species, most notably the goldfish. Given the phylogenetic diversity and wide range of lifestyles and habitats of fishes, to achieve a fuller understanding of pheromonal systems in fish, a comparative approach must be taken. Despite their distinctive reproductive strategies, which include pair-bonding and parental care, the cichlids have received surprisingly little attention in this respect. Thus, the aim of this project is to identify putative pheromones in the Mozambique tilapia (Oreochromic mossambicus; a maternal mouth-brooding cichlid) and to investigate their possible biological functions, focusing initially on reproduction. Considerable preliminary data have been accrued that suggest that female tilapia have acute olfactory sensitivity to conspecific males, and that this sensitivity is strongly correlated with sexual status. By a combination of electro-olfactogram (EOG) recording and chromatographic techniques, the aim is to identify the substances released to the water by males that evoke the strongest olfactory responses in the female. This will then allow the testing of putative biological functions of these compounds by behavioral assays ("releaser" effects) and physiological assays ("primer" effects). Although it is strongly expected that the candidate pheromones are likely to be sex hormones, or their metabolites, this strength of this approach is that it makes no prior assumptions as to their exact chemical nature. Firstly, crude chemical fractions of male body fluids (male water, urine, faeces and bile) will be tested for olfactory potency in females by EOG recording. The fractions giving the strongest responses will then be further fractionated by high performance liquid chromatography (HPLC). Once single "peaks" have been identified by HPLC, these peaks will then be collected, concentrated and again tested for olfactory sensitivity by EOG. Once those HPLC peaks giving the largest EOG responses in females have been identified, some conclusions as to their chemical identity may be drawn. This will be tested by use of pure chemical standards in the HPLC system, to see if they co-elute with the peaks obtained from the biological samples. Final chemical identification will be by gas-chromatography linked to mass spectroscopy. If avaliable commercially, olfactory sensitivity to pure compounds will be confirmed by EOG. If not, some compounds may be synthesised in the laboratory. Secondly, the effects of these compounds on the behaviour of females will be assessed initially by Y-maze experiments, and the effects on female physiology will be assessed by endocrine assays. If appropriate, assays can be utilized or developed to measure the release rates of these putatative pheromones to establish whether this is correlated with socio/sexual status. Clearly, this will depend on their chemical identity, and whether assays for these compounds are already in existance. Thus, this project forms the first stage in establishing the tilapia as an alternative model species for pheromonal studies. Not only does it have a distinct reproductive strategy, and is therefore worthy of study in its own right, but it also is reproductively active all-year-round and sexual status can be determined by external morphology, making it an ideal subject for such studies.

Reference and funding entity: POCTI/BSE/38815/2001, FCT
Duration: 1/09/2002 – 31/08/2005
Research team: Peter C. Hubbard (Coordinator), Eduardo N. Barata, Adelino V.M. Canário, Pedro A. Frade (PhD student).
Total budget: 83.000 Euro; **Funding for CCMAR**: 83.000 Euro

Title: "Chemical Communication in the Tilapia, *Oreochromis mossambicus*"
Summary and Objectives: Chemical communication is believed to play diverse and important roles in the biology of fishes. However, the number of species that have been studied in detail remains very low. Given their distinctive reproductive strategies and complex social behaviour, the cichlids have received surprisingly little attention in this respect. Over the past three years, our laboratory has made significant inroads into the understanding of chemical communication in the Mozambique tilapia, an African mouth-brooding cichlid. It is clear that this fish uses chemical signals both during reproduction and in the maintenance of social hierarchies. Thus the aim of the proposed project is to extend and embellish these initial findings, particularly with regard to the chemical cues that the females release, and answer some of the questions raised by previous research in both our and other laboratories. Firstly, the identification of putative pheromones released by pre-ovulatory females will be carried out in conjunction with IACR-Rothamsted (United Kingdom). We already know that pre-ovulatory females smell different from post-ovulatory to males, and that males behave differently towards them, depending on this olfactory cue. Our aim is to establish what these cues may be, and their likely source and routes of release. Once the likely site of phermone synthesis is established - the ovaries - we can assess the endocrine factors responsible for the regulation of pheromone production *in vitro*. This may also prove to be a convenient way to collect relatively large amounts of pheromones for identification. Thirdly, the effects of the putative pheromones on male physiology and behaviour will be investigated; we already have good evidence that female pheromones induce an increase in the urination rate of males as part of their courtship 'display' (male urine is a potent odorant to females). Lastly, we will begin to investigate how this pheromonal information is processed by the CNS using a combination of neuronal activity-dependent labelling and immunocytochemistry for the early response element *c-fos*; to where in the olfactory bulbs (and possibly beyond) this information is relayed. In the future, this will allow us to define how the pheromonal message is translated into the appropriate behavioural and physiological responses. This species has a number of advantages for this type of study; the social behaviour is well-described, males and females are easily recognisable and are reproductively active throughout the year. It is also a resilient fish and ameniable to the type of experimental manipulations outlined in this study. As such, it is an ideal introductory model for young scientists to learn how to formulate, and test, hypotheses. We think that the proposed project will establish the Mozambique tilapia as the model species for studies in chemical communication in cichlids and provide an important addition to the studies of this phenomenon in teleosts as a whole.
Reference and funding entity: POCTI/BIA-BDE/55463/2004, FCT
Duration: 1/06/2005 – 31/05/2008
Research team: Peter C. Hubbard (Coordinator), Eduardo N. Barata, Adelino V.M. Canário, Christophe Haond.
Total budget: 91 620 Euro; **Funding for CCMAR**: 91 620 Euro

Title: "Mechanisms of olfactory sensitivity to inorganic cations in teleosts"
Summary and Objectives: Without exception, teleosts are able to maintain extracellular concentrations of physiologically important ions, such as calcium and sodium, at levels

dramatically different from those of the environment. Much work has focussed on the mechanisms responsible for maintaining this differential; however, little is known about how the environmental concentrations of these ions is sensed. This is particularly important for those species that habitually encounter rapidly changing concentrations of these ions such as estuarine or migratory fish. We have recently shown that a range of different teleosts have high olfactory sensitivity to calcium and, to a lesser extent, sodium. The aim of the proposed project is to establish whether teleosts have distinct olfactory receptor mechanisms for both calcium and sodium, rather than a single 'salinity' receptor mechanism. To do this we will investigate the effects of changes in environmental ions to the olfactory sensitivity to sodium and calcium in three model species; the marine gilthead seabream (*Sparus auratus*), the esturine bass (*Dicentrarchus labrax*) and the freshwater goldfish (*Carassius auratus*). Although all three species can tollerate some change in salinity, only the bass is truly euryhaline. Furthermore, we will investigate the long-term effects of salinity changes on the olfactory sensitivity to sodium and calcium. This is important to understand whether this olfactory sensitivity is primarily linked to internal ionic homeostasis, or to inform the fish where exactly, in a fluctuating environment, it is. Lastly, we will begin to investigate where, within the CNS, this primary sensory information is relayed. This will be done by a combination of activity-dependent neuronal labelling and immunocytochemistry for the early response element *c-fos*. We expect that primary sensory input concerning food-related odorants to be processed differently from that concerning levels of inorganic cations. However, do calcium sensitive neurones project to different areas of the olfactory bulb from those sensitivie to changes in sodium? Once these questions are answered, future studies can be directed as to how these ions are detected (at a cellular and molecular level) and exactly what use the fish makes of this sensory information.
Reference and funding entity: POCTI/BIA-BCM/55467/2004, FCT
Duration: 1/01/2005 – 31/12/2007
Research team: Peter C. Hubbard (Coordinator), Eduardo N. Barata, Adelino V.M. Canário, Christophe Haond.
Total budget: 45 000 Euro; **Funding for CCMAR**: 45 000 Euro

Title: "Efeito dos factores ambientais na infecção originada por *Perkinsus atlanticus* nas populações da amêijoa *Ruditapes decussatus* – **Ambiperk**"
Summary and Objectives: The major objectives are to investigate the importance of the environmental factors on the infection of the Portuguese clam *Ruditapes decussatus* by *Perkinsus atlanticus* and the effect of this infection on the reproduction of the clam populations.
Reference and funding entity: FEDER - MARE - Programa para o Desenvolvimento Sustentável do Sector da Pesca Fundação para a Ciência e Tecnologia, 22-05-01-FDR-00020
Duration: 07/2004 - 06/2007
Research team: R. Leite, R.Afonso, P.Dias, D. Matias, S. Joaquim, M.L. Cancela (coordinator),
Total budget: 191313 euros

Title: Skeletal development, alterations and malformations during larval ontogeny of species with interest for aquaculture. Molecular, cellular and biochemical approaches - **SPARUGENES**
Objectives: To perform an integrated study to evaluate the onset of skeletogenesis and development of skeletal structures during larval ontogeny of commercially important species in the southern Peninsula and Mediterranean area (Senegal sole, Solea senegalensis; Gilthead sea bream, Sparus aurata; white sea bream, Diplodus sargo and hurta, Pagrus auriga). Expression of specific genes and accumulation of these proteins will be compared between standard rearing conditions (live food and/or equilibrated diets) and by supplying microencapsulated diets, deficient or enriched in essential micronutrients.
Reference and funding entity: Spanish funding agency CICYT

Duration: 12/2003-11/2006
Research team: Coordinator: Carmen Sarasquete CSIC/Cadiz: Participants from UALG-CCMAR, M. Leonor Cancela , P. Gavaia, JB Ortiz-Delgado, Carla Viegas
Total budget: 150.000 Euro;

Title: "Mineralization processes in marine and fresh water teleosts: function of Gla proteins (Matrix Gla and osteocalcin) – **FishDev"**
Summary and Objectives: The main objectives are to investigate the mineralization processes during development and the involvement of BGP and MGP in these processes using zebra fish and solea as fresh water and marine model organisms.
Reference and funding entity: POCTI/CVT/42098/2002 - FCT
Duration: 06/2003-2006
Research team: Coordinator: L. Cancela. Participants: P, Gavaia, D.Simes, Susana domingues, MT Dinis (consultant)
Total budget: 65.000 Euro

Title: "Effect of extracellular calcium on MGP gene expression – **SaMGP**"
Summary and Objectives: The major objectives are to investigate the function of matrix Gla protein (MGP) in the regulation of the extracellular matrix mineralization and in cell differentiation through 1) characterization of the extracellular calcium effect on the regulation of MGP gene expression by identifying calcium sending mechanisms, transcription regulatory elements and signal transduction pathways; and 2) evaluate the effect of altered MGP levels on ECM mineralization and specific gene expression.
Reference and funding entity: Fundação para a Ciência e Tecnologia, POCTI/BCI/48748/2002
Duration: 03/2003 - 02/2006
Research team: V. Laizé (coordinator), M.L. Cancela, N. Conceição, A.R. Pombinho, D. Simes, S.M.P. Marques, V. Fonseca, D. Tiago
Total budget: 99.620 euros

Title: "ARRESTED DEVELOPMENT: The molecular and Endocrine Basis of Flatfish"
Reference and funding entity: Q5RS-CT-2002-01192
Summary and Objectives: Flatfish species form a major focus of the diversification of European marine aquaculture industry. However, production has been severely hampered by biological problems in larval rearing. This project focuses on solving the major problem of arrested metamorphosis, in order to reliably control the resulting juvenile quality and production quantity. This will be achieved by addressing key questions regarding the morphological, endocrine and molecular basis of metamorphosis in the Atlantic halibut as a model species. The ultimate goal of the project is therefore to strengthen European aquaculture of marine flatfish species, an important emerging industry in many rural areas, by facilitating the production of fully functional juveniles.

Objectives
The high incidence of metamorphic abnormalities represents a serious impediment to the successful aquaculture of marine flatfish by limiting the cost-effectiveness of the juvenile production industry. The objectives of this project are to determine the biological bases for abnormalities arising during metamorphosis of a model cultured marine flatfish, the Atlantic halibut. This will be achieved by comparing normally and abnormally metamorphosing larvae in terms of differential gene expression, endocrine regulation, and biochemical and morphological transformations. This will help establish improved, cost-effective rearing techniques for the production of marine flatfish juveniles, ultimately strengthening European aquaculture of marine flatfish species, an important emerging industry in many rural, coastal areas.

Description of the work

1) The events of metamorphosis in halibut will be analysed by monitoring biochemical and morphological markers of metamorphosis. These markers are skeletal development, erythrocyte morphology, expression of troponin-T isoforms, production of gut enzymes, and expression of immunoglobulins.
2) Differential gene expression will be analysed by screening of a gene microarray based on genes induced during metamorphosis, This will allow high throughput analysis of an extensive panel of genes involved in metamorphosis.
3) Endocrine regulation of metamorphosis will be analysed in terms of hormone levels and hormone responsiveness. Tissue and plasma levels of thyroid hormones, cortisol, growth hormone and IGF-1 during metamorphosis will be determined by RIA. Prolactin expression will be monitored by assaying its mRNA. The genes encoding receptors for prolactin, cortisol and growth hormone will be cloned. The temporal and spatial expression of these genes, along with the already-cloned thyroid hormone receptor will be analysed during metamorphosis by competitive RT-PCR and in situ hybridisation.The role of individual hormones during metamorphosis will be further investigated through hormone treatment experiments on pre-metamorphic and metamorphosing larvae.
4) Abnormally metamorphosing larvae will be collected, grouped according to type of abnormality (arrest of metamorphosis, pigmentation defect, inappropriate eye migration etc). These larvae will be compared with normally metamorphosing larvae from the same culture in terms of expression of biochemical and morphological markers of metamorphosis, differential gene expression, expression of hormone receptors and hormone content. This will indicate the underlying molecular and/or endocrine bases of the various abnormalities.
5) In vivo treatments with hormones and nutritional supplements will be carried out to establish functional relationships.
Milestones
A staging scheme for metamorphosis, based on biochemical, morphological endocrine and genetic markers. A ""metamorphic"" microarray of 700 genes to identify endocrine and molecular basis of metamorphic arrest. Larval treatments with hormones, iodine arid selenium to establish functional relationships between nutritional factors, endocrine axes and metamorphosis, establishing ""functional feeds"" and scientific tools for effective metamorphic larval aquaculture
Duration: 1/10/2002 – 31/09/2005
Research team: D. M. Power
Total budget: Euro; **Funding for CCMAR**: 217 536 Euro

Title: “Bridging genomes: an integrated genomic approach toward genetic improvement of aquacultured fish species”
Reference and funding entity: EC Q5RS-CT-2001-01797
Summary and Objectives: The project will use a far reaching but parsimonious approach to:

- transfer genetic information from model organisms to commercial species;
- transfer technology and know-how from leading laboratories in genome analysis and mapping to more classical fish genetics laboratories, as well as knowledge from and interesting biological model, the sea bream, in the opposite direction;
- bridge the gaps in maps by merging physicals and linkage maps;
- bridge the distance between research and industry;
- integrate evolutionary theory and modern technology to generate an applied endpoint;
- integrate genome maps of various teleost with data from higher vertebrates, thanks to the high potential of comparative mapping.

The project workplan is centered round the application of modern biotechnological methods to aquaculture. It is subdivided into 7 main workpackages, each of which is co-ordinated by the partner with the relevant expertise, and the completion of which will contribute to the attainment of the project objectives.
The workpackages articulate with each other and can be subdivided into 3 main groups according to the nature of the methods they use:

i) molecular biotechnology, which includes methodologies for generating mapping panels for the linkage map (WP1) and HAPPY mapping (WP2), a highly automatable new method

for physical mapping, genotyping the linkage map (WP3) and HAPPY map (WPS) by use of high throughput automated methods, and isolating STS markers for HAPPY mapping (WP4);
ii) conventional genetic; the tools generated in WPI-5 will be used to screen sea bream (Sparus aurata) generated in a breeding program on a SME fish farm. This will result in the transfer of molecular biotechnological methodologies to conventional genetics and the implementation of technology transfer from science to industry, an;
iii) the final workpackage which will run simultaneously with the other project tasks is the analysis of the extensive data which will be generated by the various workplans of the project. Bioinformatics will be essential for the handling and interpretation of the data and for its successful dissemination, in order that the project can have a maximum impact in the fields of aquaculture research, fish genetics, and comparative mapping.

Duration: 1/11/2001 – 31/10/2005
Research team: D. M .Power, A. Canário.
Total budget: Euro; **Funding for CCMAR**: 211 906 Euro

Title: "Calcium, the backbone of fish culture: importance in skeletal formation, reproduction and normal physiology – **Fishcal**"
Summary and Objectives: Egg and larval viability in sea bream culture is still low, as a consequence of mortalities and a high incidence of skeletal deformities (dystrophies). Dystrophies are not always immediately apparent, leading to wasteful use of food, energy, space and human resources. Abnormal cartilage growth and calcification are key features of skeletal deformities. Parathyroid hormone-related protein (PTHrP), recently identified as a hypercalcaemic hormone in fish, appears to mediate ossification. Calcium is also essential in many other physiological processes, such as reproduction and growth. The project will: i) establish the relative contribution of the diet and the environment to calcium balance; ii) determine the part played by PTHrP in larval development, growth and vitellogenesis; iii) identify genes regulated by PTHrP and iv) generate guidelines for the use of calcium in sea bream husbandry.
Reference and funding entity: European comission
Duration: 11/2001-4/2005
Research team:
Total budget: Euro; **Funding for CCMAR**: Euro

Title: Identification of Sex Pheromones from the Anal Gland of Male Blennies, *Salaria pavo* and *S. fluviatilis* (Pisces: Blenniidae)
Summary and Objectives: *Salaria pavo* is small bottom living fish in the littoral zone of the Mediterranean and adjacent Atlantic coast. The closely related freshwater species, *S. fluviatilis*, inhabits rivers and lakes in the vicinity of the Mediterranean. In both species, the mating system is promiscuous. Males occupy holes or crevices in rocks where females come to spawn and the males subsequently guard the eggs. These are a good model fish species to investigate specialization in sex pheromone production. The males develop anal glands from the epidermis of the first two rays of the anal fin concurrent with development of the gonads. The research team has previously shown that the anal gland of *S. pavo* is a source of substances that attract pre-ovulatory females and promotes male reproductive success. This suggests that male blennies are "active signallers" in contrast with known pheromone systems in teleosts, where receivers are "chemical spies" detecting gonadal steroids or prostaglandins passively excreted by females into the water *via* the urine or gills.
The proposed work aims to identify the chemical structures of sex pheromones in both species. The marine origin of *S. fluviatilis* is well established and, as with *S. pavo*, it is plausible that the anal gland has a pheromonal function in female attraction. If so, one would expect the pheromonal components of the two species to have similar or closely related chemical structures. In addition, the inclusion of *S. fluviatilis* in the project will

simplify the chemical identification, since recording of electro-olfactograms (EOG) is technically easier in freshwater, and can be combined with chromatographic separation of anal gland-produced substances.
The research will involve: 1) testing a pheromonal function for the anal gland of males *S. fluviatilis* in female attraction, through behavioural assays in the laboratory; 2) evaluation of the specificity of pheromonal action through test of the behavioural activity of substances from the anal gland of male *S. fluviatilis* and the marine fish, *S. pavo*, on females of the other species; 3) solid phase extraction of substances released by the anal gland of both species, fractionation of extracts by vacuum distillation and chromatographic techniques, and evaluation of biological activity of each fraction through behavioural assays and EOG recordings; 4) chemical identification of active substances, and synthesis of putative pheromones; 5) confirmation of biological activity of synthesised chemicals.
This research project will likely provide empirical evidence to fill a gap in the current leading hypothesis on the evolution of fish sex pheromones. Known pheromonal systems in teleosts indicate that males are "chemical spies" of information excreted by females. Evidence of true chemical communication is lacking, where senders should have evolved a specialization in the way they produce and release a signal. The results will also add to the knowledge on *S. pavo* biology, grounding management strategies of the populations in the nature park of Ria Formosa (Algarve).
Reference and funding entity: POCTI/BSE/45843/2002, FCT
Duration: 1/02/2004 – 31/01/2007
Research team: Eduardo N. Barata (Coordinator), Peter C. Hubbard, Adelino V.M. Canário.
Total budget: 59.706 Euro; **Funding for CCMAR**: 59.706 Euro

Title: "Development of Virtual Learning Environment in Environmental Science, with Online Re-usable Interactive modules for remote users in marine pollution and ecology, with self learning languages packages in English, Greek, Portuguese and Swedish – **ORION**"
Summary and Objectives: ORION will develop a state-of-the-art Virtual Learning Environment, housing a Resource Repository for a 4-language glossary, self-instruction language modules in English, Greek, Portuguese and Swedish, with environmental science course materials (marine pollution studies, ecology, marine biodiversity, etc.) suitable for the vocational and tertiary sectors at various levels, prepared by university departments and research organisations in Greece, Ireland, Portugal and Sweden. The major goal of ORION is the development of a powerful state-of-the-art ICT learning/teaching tool, to be used within a Virtual Learning Environment and delivered by means of a Distributed Network, in the subject area of the marine environment (pollution studies, ecology, biodiversity, aquaculture, etc.) relevant to both the vocational and tertiary sectors at various levels. This is an area of great importance, subject to increasing legislative and regulatory demands affecting a range of coastal zone users and decision-makers. These multi-disciplinary materials, prepared by top-level trainers in native English, Swedish, Portuguese and Greek, will be developed as innovative re-usable learning objects which can be shared and re-used by teachers and target group users situated in remote areas of Europe: Stockholm in the North, Cork in the north-west, Portugal in the south-west and Greece in the south.
To underpin the training in language which will be needed to maximise the benefits from this innovative methodology, an online marine environmental glossary in English, Greek, Portuguese and Swedish will be created, to form a major resource freely available to partners only, with accompanying modules in ESP (environmental science) and basic Greek, Portuguese and Swedish language modules, to reflect the needs of the target users.
Reference and funding entity: European Community Programme LEONARDO DA VINCI, Community Vocational Training Programme Project nº EL 2001 BP LA 114443**,**
Duration: April 2002 – April 2005
Total Funding: 404.672 Euros

Partners: FEAP (Federation of European Aquaculture Producers), IMBC, Crete, Greece, EKTHE (National Centre for Marine Research, Athens), TEREUS, SA, Athens, Department of Zoology, University of Cork, Ireland, AQUALEX Multimedia Consortium Ltd Ireland, Centre for Marine Sciences, University of Algarve, Portugal, Department of Systems Ecology, University of Stockholm, Sweden

Title: "Hormones and life-history trade-offs and plasticity: a study on alternative reproductive tactics in blenniid fish"
Reference and funding entity: Fundação para a Ciência e a Tecnologia POCTI/BSE/38395/2001
Summary and Objectives: Diversas espécies apresentam histórias vitais alternativas. Contudo, os mecanismos causais subjacentes à plasticidade das histórias vitais e aos "trade-offs" (e.g. investimento na reprodução presente vs reprodução futura) só recentemente têm sido tema de investigação.
Numa população de Salaria pavo (Blenniidae) da Ria Formosa (Portugal) ocorrem dois tipos de histórias vitais alternativas. De entre os machos recrutados no próprio ano, os mais pequenos reproduzem-se como "sneakers", enquanto os maiores continuam a crescer, apenas se reproduzindo na segunda época de reprodução, no ano seguinte, como "nest-holders". Assim, nesta população existem dois tipos de machos sexualmente activos: machos mais velhos e maiores que defendem ninhos e cuidam dos ovos (CT »14 cm; idade ³ 2 anos) e machos mais pequenos e mais jovens (CT » 10 cm; idade < 1 ano) que imitam o comportamento e a coloração nupcial das fêmeas na tentativa de se aproximarem dos machos que defendem ninhos e fertilizarem parte dos ovos (Gonçalves *et al*. 1996). Os machos que se comportaram como "sneakers" durante a sua primeira época de reprodução podem tornar-se "nest-holders" em épocas de reprodução subsequentes. Deste modo, o comportamento de "sneaking" parece ser uma estratégia condicional nesta espécie.
Neste projecto tencionamos investigar os mecanismos causais que permitem a expressão das histórias vitais alternativas acima mencionadas e dos "trade-offs" envolvidos. Os principais objectivos são os seguintes: 1) caracterizar as vias ontogenéticas alternativas e a plasticidade do comportamento de acasalamento dos machos jovens (i.e. classe etária 0+/1), através de um programa de marcação e recaptura intensivo e de longa duração (3 anos), que recorrerá à implantação de marcas magnéticas; 2) caracterizar os perfis hormonais dos diferentes tipos de machos recém recrutados (i.e. "sneakers" vs. machos não reprodutores), assim como dos "nest-holders" que servirão de referência para os parâmetros de reprodução. Proceder-se-á à quantificação das seguintes hormonas: esteróides sexuais em circulação (e.g. testosterona, 11-Cetotestosterona); ii) neuropéptideos da família da prolactina (e.g. hormona do crescimento, somatolactina, prolactina); iii) gonadotrofinas (GtH I e II); e iv) GnRH e AVT na área pré-óptica; 3) testar experimentalmente os efeitos do tamanho relativo, da densidade e do estatuto social nas decisões da história vital dos jovens machos, manipulando estas variáveis em grupos mantidos em cativeiro e avaliando o impacto da manipulação experimental nos parâmetros referidos em 1); 4) testar de que forma a presença de machos grandes, defensores de ninhos afectam a direcção da via ontogenética dos machos jovens, avaliando o efeito da exposição dos machos recém recrutados aos machos defensores de ninhos.
O conhecimento da biologia reprodutora constitui um excelente modelo para o estudo integrado dos mecanismos causais da plasticidade sexual e das histórias vitais dos teleósteos.
Duration: 2002-2004
Research team: Rui Oliveira (Ispa- coordinator), A. Canário, D. Power
Total budget: 103.080 Euro; **Funding for CCMAR**: Euro

Title:" Isolation of carotenoid-overproducing *Dunaliella salina* strains. **OVERCAROTEN**."
Summary and Objectives: To isolate novel strains of microalgae, namely *D. salina* and *H. pluvialis able to accumulate higher levels of carotenoids at early stages of growth.* This will be accomplished by several strategies: 1) generation and screening of mutants by chemical mutagenesis and carotenoid biosynthesis inhibitors; and 2) metabolic engineering. The latter strategy will be implemented by the development of genetic transformation procedures and

expression of homologous and heterologous genes associated with the carotenogenesis in these microalgae.
Reference and funding entity: FCT, PDCTM / MAR / 15237 / 99
Duration: February 2002 – February 2005
Research team: CCMAR: João Varela, Vanessa Duarte, Sacha Coesel, Nuno Henriques. **INETI:** Rui Mendes e Luísa Gouveia; **NÉCTON:** João Navalho e Vítor Duarte; **ESB-CU:** Rui Morais.
Total budget: 175000 Euros**. Funding for CCMAR:** 77935 Euros.

Title: "Microalgae as cell factories for chemical and biochemical products. **ALGINET**."
Summary and Objectives: Microalgae are microscopic photosynthetic organisms that form the base of the food chain. They have long been proposed as possible "cell factories" for obtaining chemical and biochemical substances. There has been considerable interest in the field in recent years and a number of small companies have been founded to market microalgae and microalgal products. The main products are microalgae used as animal feed or as human health supplements, but a number of companies are marketing products directly obtained from microalgae (e.g. 3R,3'R-zeaxanthin, an important carotenoid). This proposed thematic network aims to overcome these problems by improving communication between researchers and manufacturers in the microalgal field, and by encouraging further development in the field. Its main goals are: 1) Improve communication between workers in the field of microalgae; 2) Focus the direction of European research, to open new markets for microalgal products; 3) To provide a standard reference portal for workers in the field; 4) Enable rapid dissemination of research results, to speed the uptake of new technologies; 5) To attract new interest in the field; 6) To develop an ongoing 'virtual institute' model and lay the groundwork for future RTD projects.
Reference and funding entity: European Union, QLK3-CT-2002-02132
Duration: February 2003 – February 2005
Research team: CCMAR: João Varela Ana Ramos e Ana Rita Marques.
Total budget: 1221878 Euros**. Funding for CCMAR:** 28656 Euros.
Site: www.algi-net.org

Title: "Nutritional requirements and feeding of blackspot seabream (*Pagellus bogaraveo*), a new species for aquaculture – **GORAZ**"
Summary and Objectives: In order to ensure a sustainable growth of the Portuguese mariculture, it is necessary to diversify the offer of cultivated species to avoid market saturation, competition among producers and to increase the efficacy of production facilities, namely hatcheries. The marine teleost, blackspot seabream (*Pagellus bogaraveo*), has a high market price and is considered as a strong candidate species for intensive aquaculture in Atlantic coasts. Up to now, studies with blackspot seabream under captivity are extremely scarce and have dealt mainly with the control of reproduction, larvae and juveniles cultivation techniques. To our knowledge, blackspot seabream has been fed with diets developed for other marine fish, namely gilthead seabream, and no available literature data exists on the specific nutritional requirements of this species. Therefore, the overall objective of this project is to contribute towards a better knowledge of the nutritional requirements and feeding strategies of the larvae and juveniles of blackspot seabream. Concerning the larvae, studies will cover: 1) optimisation of feeding strategies with live preys; 2) evaluation of precocious feeding strategies with micro-particulate diets; 3) optimisation of the dietary composition of micro-particulate feeds (i.e. dietary lipid, protein and energy level and source, adequate dietary PUFA level and DHA/EPA ratio). Regarding the juveniles, studies will evaluate: 1) the dietary protein requirements; 2) the relative potential of proteins, fats and carbohydrates as energy donors; 3) the optimal dietary DP/DE ratio. Given the economic importance and ecological implications of man-made feeds and feeding in aquaculture, the development of nutritionally balanced and environmental friendly diets is of utmost importance for a future establishment of blackspot seabream as a consolidated species in intensive aquaculture.
Reference and funding entity: Fundação para a Ciência e Tecnologia, POCTI 39239/2001.
Duration: Out 2002 – Set 2005

Research team: CCMAR: Maria Teresa Dinis, Luis Conceição, Florbela Soares, Laura Ribeiro; CIIMAR: Emidio Gomes, Paulo Rema, Luisa Valente; DAM-SRP da Madeira: Carlos Andrade, Nuno Gouveia

Total budget: 100.000 Euros **Funding for CCMAR**: 15.081 Euros

Title: "Probiotics and immunemodulation in marine fish larvae and juveniles – **PROBIMU**"
Reference and funding entity: FCT Project nº 38781/BSE/2001
Duration: Mar 2002 – Feb 2005
Summary and Objectives: Most bacteria causing disease in marine fish are opportunistic pathogens that are present as part of the normal seawater microflora. Environmental stress may weaken the immune system of the larvae and allow opportunistic pathogenic bacteria to invade the fish tissues, leading to disease.
Fish larvae drink water soon after hatching, and ingest bacteria associated with the water. Members of the "pioneer" microflora established in the larval gut may acquire a competitive advantage and become part of a persistent flora at the juvenile stage. Therefore, the early colonisation with non-pathogenic bacteria seems to be essential and has been shown to reduce mortalities after infection with pathogenic bacteria. Beneficial effects of probiotics in growth promotion and disease prophylaxis have also been reported. During the early life stages, fish have a very limited specific immune system, making difficult the use of vaccines. Successful use of immunostimulants in marine fish larviculture has been reported in recent years. Use of immunostimulants may aid the fish larvae to overcome stressful situations and critical stages during the early phase of rearing. Immunostimulants may act, either by stimulation of the non-specific mechanisms, or by acceleration of the development of the specific immune system.
This project aims at improving growth and survival of larval and juvenile marine fish using both probiotics and immunostimulants, as well as to find possible interactions between the two approaches. The Senegal sole (*Solea senegalensis* Kaup) and gilthead sea bream (*Sparus aurata* L.) will be used as models. In particular, it is intended to verify to what extent the immune system can be influenced by potential immunostimulants, and whether growth and survival can be improved by manipulation of the species composition of the gut microflora. The project also intends to study the effect of exposure of fish larvae and juveniles to virulent pathogens after immune stimulation and/or modulation of the water microflora.
Ontogeny of the immune system organogenesis, non-specific cellular immunity and humoral immunity will be characterised in sole. Candidate probiotic strains will be isolated from the larval gut microflora based mainly on their inhibitory activity on virulent bacteria. The relative virulence of different pathogenic bacterial strains will be determined through challenge tests.
The effects of immunostimulants and selected probiotics on the development of the immune system, on the immune response, and on fish growth and survival will be assessed. This will be carried out by using standard microbiological, immunological and physiological techniques, both in normal conditions and when exposed to virulent pathogens.
Research team: CCMAR: Maria Teresa Dinis, Luis Conceição, Pavlos Makridis; IBMC: Pedro Rodrigues.
Total budget: 71.500 Euro; **Funding for CCMAR**: Euro

Title: "The Role of Olfaction in the Feeding Behaviour of *Solea senegalensis* – **SOLFACTO**"
Summary and Objectives: Food consumption is the primary determinant of growth in fish. Many fish species, in particular those with nocturnal activity and/or in habitats with frequent high water turbidity, rely mostly on chemo-sensory mechanisms for food detection and location. In different species, feeding behaviour is triggered by different chemical substances, some of which may act as attractants via olfaction, and others may act as promoters or enhancers of food consumption via both olfaction and gustation. In general, free amino acids, nucleotides, nucleosides and quaternary ammonium bases have been identified as feeding stimulants in some species.
The Senegal sole (*Solea senegalensis*) is a good model species to investigate olfactory mechanisms underlying feeding behaviour, due to its feeding strategy, and a well-developed olfactory system accessible to electrophysiological recordings. The central aim of this project is to

identify substances released by natural food sources that act as olfactory cues involved in food-search behaviour, and ingestion of food. This objective will be achieved by an integration of electrophysiological, behavioural and food ingestion measurements in response to candidate olfactory stimuli.
A method for the electrophysiological recording from the olfactory system of juvenile sole will be employed, based on electro-olfactogram and/or multi-unit olfactory nerve recordings. This will give quantitative measurements to define relative olfactory sensitivities to various chemical stimuli. Stimuli to be tested include water containing live natural food items (e.g. polychaetes), crude filtered macerates of food items, chromatographic fractions of water containing the food items and of its macerate, and a range of amino acids, nucleosides and nucleotides. The most potent olfactory stimuli identified will be used in behavioural assays.
Two types of behavioural assays will quantify how a given chemical stimuli affect the patterns of food-search behaviour in juvenile sole. The first will employ Y-maze aquaria to quantify the end result of food-search behaviour. The second assay will quantify specific behavioural acts during food-search behaviour observed in single fish. In both assays, sole behaviour will be videotaped for a fixed period of time and subsequently analysed for pre-defined behavioural responses. The strength of these responses will be related to stimulus quality and intensity.
Food consumption measurements and growth trials will be conducted to test the effect of olfactory stimuli shown to improve food-search behaviour on the ingestion of inert food pellets by juvenile sole. Measurement of ingestion will be obtained by a method employing X-radiography.
Finally, we will examine whether olfactory stimuli that affect feeding behaviour of juvenile sole also act in stimulating behavioural elements associated with feeding in early juveniles (after metamorphosis) during the weaning period.
Ultimately, the project will identify the chemical cues that are important in the initiation of food-search behaviour, food ingestion, and therefore growth. This may be the ground for future technological development of new inert food diets that enhance the farming of this commercially important species.
Reference and funding entity: POCTI/CVT/38831/2001, FCT
Duration: 1/01/2003 – 31/12/2005
Research team: Eduardo N. Barata (Coordinator), Peter C. Hubbard, Adelino V.M. Canário, Luis Conceição, Pavlos Makridis, Maria T. Dinis.
Total budget: 100.000 Euro; **Funding for CCMAR**: 100.000 Euro

Title: "The underlying mechanisms of the effect of microalgae on the early life stages of fishes – **MICROALGAE**"
Summary and Objectives: The benefical role of microlagae on the development of marine fish larval is widely reported, however the mechanisms are still poorly understood. This project aims to understand a bit further how microlage affect marine fish larvae early stages. Two species of microalgae (Tetraselmis chui and Isocrhysis galbana), a commercial microalgae concentrate (Fitobloom®, Necton) and clear water, will be used as treatments. Sea bream and sole were the marine fish species used in this study since they are commonly used for aquaculture in the southern Europe. This project intends to analyse: 1) the effect of microalgae on larval ontogeny, growth and survival, biochemical composition and larval condition; 2) the effect of microalgae on the activity of digestive enzymes and some key enzymes of intermediary metabolism; 3) the effect of microalgae on food intake; and 4) the effect of microalgae on the modulation of the intestinal microflora. The project expects to increase the knowledge on the effect of microalgae on fish larval development, which will contribute to obtain higher quality aquaculture products.
Reference and funding entity: Fundação para a Ciência e Tecnologia, POCTI/BSE/37378/2001
Duration: Out 2002- Set 2005
Research team: CCMAR: Maria Teresa Dinis, Laura Ribeiro, Luis Conceição, Pavlos Makridis, Rui Rocha, Pedro Cação, João Sendão; ICETA: Emídio Gomes.
Total budget: 67,000 Euro; **Funding for CCMAR**: 59518.02 Euro

Title: "Minimization of the effects of stress in senegal sole through amino acid supplementation - **STRESSAA**"

Summary and Objectives: Stressful conditions are known to cause growth suppression in cultured fish, either by impacts on appetite reduction, a stimulated catabolism, or a combination of both. As growth is essentially protein deposition, its optimisation depends on the understanding of protein and amino acid (AA) metabolism. The relative balance of the different metabolic pathways involved in AA metabolism is affected by the physiological condition of the animal. Thereby, stressful husbandry conditions do affect AA requirements. The central objective of this study is to contribute to a better understanding of the metabolic processes impinging on amino acid requirements of animals when they are exposed to stress situations. Post-larval and juvenile Senegal sole (Solea senegalensis) will be used as model species, because it is a species resistant to stress in terms of survival and also because it is a species of importance to the Portuguese marine aquaculture industry. It is intended to verify to what extent the amino acid metabolism of fish change when fish are under stress situations, and also whether the metabolic and growth depression effects of stress can be reduced by AA supplementation. The project will involve a first part were the effects of selected stressful husbandry conditions on growth and AA metabolism will be assessed in post-larvae and juvenile sole. This will involve the study of AA metabolism through different angles and methodologies: AA utilisation will be studied using tracer studies for individual AA, nitrogen balances and plasma free AA levels; food consumption will be determined using 14C as tracer for post-larvae and feed with glass beads plus x-rays for juvenile fish; stress condition will be ascertained by measuring plasma levels of cortisol, lactate and glucose; and the relative activity of the different intermediary metabolism pathways will be assessed through the determination of the activities of different enzymes. The second part of the project will verify to what extent the negative effects of stressful husbandry conditions on amino acid metabolism and retention can be minimized through supplementation of the diets with individual amino acids. Post-larvae and juvenile fish will be reared under selected acute and chronic stressful conditions based on the results of the first part of the project. Diets will be supplemented with individual key AA depending also on the results of the first part of the project. The same methodologies as before will be used to evaluate the results. Ultimately, this project expects to contribute to: 1) clarify the relation between stressful husbandry conditions and AA metabolism; 2) understand to what extent stress can affect animal growth and AA requirements; and 3) ascertain whether stress effects on growth and susceptibility to disease can be minimized through AA supplementation.

Reference and funding entity: Fundação para a Ciência e Tecnologia, POCTI/CVT/49324/2002

Duration: Fev 2005- Jan 2007

Research team: Luis Conceição, Cláudia Aragão, Maria Teresa Dinis.

Total budget: 50,000 Euro; **Funding for CCMAR**: 50,000 Euro

Title: "Understanding the regulation of the digestive function on marine fish larvae - **DIGFISH**"

Summary and Objectives: Scarce information exists concerning the digestive function of marine fish larvae. However, understanding the digestive function of fish larvae and the mechanisms they use to regulate this function at different stages of development is extremely important. This information will allow to stimulate food intake, to adequate diet composition to a specific stage of development and related them with food assimilation rates. Therefore, the knowledge of these mechanisms will contribute to enable marine fish larvae fed with microdiets to achieve growth and survival rates identical to live food.

To achieve this goal it is important to study the ontogeny of the neuro-endocrine system associated to marine fish larvae digestive tract, which in resemblance with other larval systems is poorly developed at first feeding although functionally adapted to this stage of development. According to existing bibliography, different periods can be identified on the neuro-endocrine system ontogeny, reflecting developmental differences in the regulation mechanisms of larval digestive function. The immunohistochemical methods are essential in this type of studies, since they allow the identification and location of nervous fibres and neuropeptides, although the latter only at a semi-quantitative level.

Some studies reported the importance of visual and chemical stimuli on the increase of food intake, either with live food or microdiets. Although an increase in pancreatic enzymes was reported, no relation was established with the regulation of the digestive function. The small dimensions of fish larvae difficult the use of standard methodologies for this kind of studies. The use of a new methodology will allow the quantification of cholecystokinin (CCK) secretion on individual larvae. With this method the influence of different stimuli on digestive function will be assessed, especially the pancreatic function that is essential during the larval stages of marine fishes. In parallel, the use of labelled food, based in another new methodology, will allow to quantity the effect of different stimuli on food ingestion and assimilation by fish larvae that will allow the evaluation of digestive function efficiency at different stages of development.
Reference and funding entity: Fundação para a Ciência e Tecnologia, POCTI/CVT/58790/2004
Duration: 2005- 2008
Research team: CCMAR: Laura Ribeiro, Maria Teresa Dinis, Deborah Power, Cláudia Aragão.
Total budget: 73056 Euros

Title: "Physiological importance and metabolism of aromatic and sulphur AA during fish ontogeny - **SULFAAR**"
Summary and Objectives: The major fate of amino acids (AA) is towards protein synthesis, but studies showed that the determination of the AA requirements should consider not only the AA profile of the proteins being synthesised, but also which AA are used for energy or for other metabolic purposes. Some AA are involved in the synthesis of other compounds of physiological importance, therefore a better understanding of its physiological role and metabolism deserves special attention. Among them, sulphur and aromatic AA may seem to have a special importance during the fish ontogenesis. The main objective of this study is to acquire a better knowledge on the physiological importance and metabolism of these AA during the early life stages of fish, focusing especially on taurine and tyrosine. Several questions regarding these AA need to be clarified and intend to be study within this project. Three model species will be used in this project: toadfish, which is a marine species with demersal eggs, seabream, and sole, which are marine species with pelagic eggs. Furthermore, sole and seabream are species with or without a marked metamorphosis, respectively. The first part of this project intends to analyse the aromatic and sulphur AA metabolism along development and to compare this metabolism in species with dermersal and pelagic eggs. This will involve the analysis of the free AA profile in eggs and larvae of the three model species. In this first part, the larvae will also be tube-fed with radio-labelled precursors of taurine and tyrosine and is intended to verify if the pathways for the biosynthesis of these AA are available in young fish stages. The second part of this project intends to better understand the effects of dietary taurine or tyrosine supplementation in growth, metamorphosis, and AA metabolism of fish larvae. This will be done by conciliating traditional studies on AA metabolism, involving the rearing of the species and the analysis of several paramenters, with more recent techniques, such as the tube-feeding of radio-labelled AA, in order to analyse differences in AA utilisation. Larvae will be reared according to standard procedures and using diets supplemented or not with taurine or tyrosine. The first trials will analyse the effects of taurine supplementation on growth performance of the three model species. The second trials will analyse the effects of tyrosine supplementation on metamorphosis of fish species with and without a marked metamorphosis process, using sole and seabream as model species. For both experimental trials, the effects of AA supplementation on AA utilisation will be analysed. Fish receiving or not a dietary taurine or tyrosine supplementation will be tube-feed 35S- or 14C-labelled AA and the fate of this AA will be followed in the fish. This project will ultimately result in a better understanding of the AA requirements during fish ontogenesis, which will have an impact in the aquaculture industry.
Reference and funding entity: Fundação para a Ciência e Tecnologia, POCTI/CVT/60176/2004
Duration: 2005 - 2008
Research team: CCMAR: Cláudia Aragão, Laura Ribeiro, Luís Conceição, Maria Teresa Dinis.
Total budget: 91,500 Euro; **Funding for CCMAR**: 91,500 Euro

Title: "Optimização da reprodução do linguado (*Solea senegalensis*)- **REPROSOL**"

Summary and Objectives: The investigation of new species for aquaculture, such as the sole (*Solea senegalensis*) could offers potential development for many coastal regions along the Mediterranean belt (Dinis et al, 1999). However the mass production of this species has not yet been achieved. One of the major problems in the culture of this species is the control of reproduction in captivity and the production of regular high quality spawns. It is well known that environmental factors such as temperature and photoperiod as well as nutrition, play an important role in fish reproduction. However, little is known about how those factors can be controlled and evaluated, in order to produce high quality gametes in sole fish. Egg quality is a specific requirement for the production of healthy larvae. The aim of the present project is evaluate the parameters (zootecnical and feeding plan) that can control reproduction in sole maintained in captivity. Several sperm and egg parameters (biochemical and physiological) will be determined and analysed for correlations with fertility and hatching rates.
It is proposed to achieve these objectives:

a. Parameters optimization on the broodstock reproduction
b. The importance of controlled husbandry conditions on the maturation and emission of gametes, as well as in the production of high quality spawns.
c. Characterize the influence of husbandry conditions, such as temperature, photoperiod and nutrition on gametes and egg quality.
d. Effect of the hormonal induction in the broodstock reproduction
e. Identification and caracterization of the feeding plan for sole
f. Characterize and identify quality in gametes and spawns

Reference and funding entity: MARE- 22-05-01-FDR-00026
Duration: 2004 - 2006
Research team: CCMAR: Florbela Soares, Maria Teresa Dinis, Elsa Cabrita.
Total budget: 224.507 Euro

Completed in 2004

Title: “Chemical Identification and Functional Roles of Reproductive Pheromones in the Tilapia, *Oreochromis mossambicus*”
Summary and Objectives: That pheromones play important roles in many aspects of fish biology is beyond doubt. However, the most complete evidence do date has been obtained from a few well-studied species, most notably the goldfish. Given the phylogenetic diversity and wide range of lifestyles and habitats of fishes, to achieve a fuller understanding of pheromonal systems in fish, a comparative approach must be taken. Despite their distinctive reproductive strategies, which include pair-bonding and parental care, the cichlids have received surprisingly little attention in this respect. Thus, the aim of this project is to identify putative pheromones in the Mozambique tilapia (Oreochromic mossambicus; a maternal mouth-brooding cichlid) and to investigate their possible biological functions, focusing initially on reproduction. Considerable preliminary data have been accrued that suggest that female tilapia have acute olfactory sensitivity to conspecific males, and that this sensitivity is strongly correlated with sexual status. By a combination of electro-olfactogram (EOG) recording and chromatographic techniques, the aim is to identify the substances released to the water by males that evoke the strongest olfactory responses in the female. This will then allow the testing of putative biological functions of these compounds by behavioral assays ("releaser" effects) and physiological assays ("primer" effects). Although it is strongly expected that the candidate pheromones are likely to be sex hormones, or their metabolites, this strength of this approach is that it makes no prior assumptions as to their exact chemical nature. Firstly, crude chemical fractions of male body fluids (male water, urine, faeces and bile) will be tested for olfactory potency in females by EOG recording. The fractions giving the strongest responses will then be further fractionated by high performance liquid chromatography (HPLC). Once single "peaks" have been identified by HPLC, these peaks will then be collected, concentrated and again tested for olfactory sensitivity by EOG. Once those HPLC peaks giving the largest EOG responses in females have been identified, some conclusions as to their chemical identity may be drawn. This will be tested by use of pure chemical standards in the HPLC system, to see if they co-elute with the peaks obtained from the biological samples. Final chemical

identification will be by gas-chromatography linked to mass spectroscopy. If avaliable commercially, olfactory sensitivity to pure compounds will be confirmed by EOG. If not, some compounds may be synthesised in the laboratory. Secondly, the effects of these compounds on the behaviour of females will be assessed initially by Y-maze experiments, and the effects on female physiology will be assessed by endocrine assays. If appropriate, assays can be utilized or developed to measure the release rates of these putatative pheromones to establish whether this is correlated with socio/sexual status. Clearly, this will depend on their chemical identity, and whether assays for these compounds are already in existance. Thus, this project forms the first stage in establishing the tilapia as an alternative model species for pheromonal studies. Not only does it have a distinct reproductive strategy, and is therefore worthy of study in its own right, but it also is reproductively active all-year-round and sexual status can be determined by external morphology, making it an ideal subject for such studies.
Reference and funding entity: POCTI/BSE/38815/2001, FCT
Duration: 1/09/2002 – 31/08/2004
Research team: Peter C. Hubbard (Coordinator), Eduardo N. Barata, Adelino V.M. Canário, Pedro A. Frade (PhD student).
Total budget: 83.000 Euro; **Funding for CCMAR**: 83.000 Euro

Title: "Design and development of commercial scale farming technologies for sole – **SOLEMATES**"
Summary and Objectives: The overall objective of this project is to design and develop technologies for commercial scale farming of the sole species Solea solea and Solea senegalensis. The project will include the development of a commercial scale fingerling production system, namely optimisation of weaning zoo technical conditions and feeding regimes. It will obtain data on feeding and growth of both species of sole, including a comparison of growth potential in different conditions between the two, and development of an optimised feed for sole. This project will also work in the development of ongrowing and feeding systems suited for different areas within Europe, more specifically: earth ponds, shallow raceways, circular tanks and terraces.
Reference and funding entity: European Community - CRAFT Project Q5CR-2002-71039
Duration: Nov 2002 – Out 2004
Research team: CCMAR: Maria Teresa Dinis, Luis Conceição, Florbela Soares, Sofia Engrola; CIIMAR: Emidio Gomes, Paulo Rema, Luisa Valente; RIVO (NL): Andries Kamstra, Edward Schram; Akvapna Niva (NO): Patrick White, Albert Imsland; DVZ-CLO (BE):Daan Delbare.
Total Budget: 1.801.857 Euros **Funding for CCMAR:** 165.480 Euros

Title: "The use of RMBC's for bacterial management in marine larval fish – **RMBC**"
Reference and funding entity: European Community - CRAFT Project Q5CR-2002-72221
Duration: Jan 2003 – Dec 2004
Summary and Objectives: The challenge of this study consists in screening mixtures of bacteria at specific stages of the larval rearing where bacterial interference may be critical. These bacterial communities, referred to as RMBC's (Revolving Multifunction Bacterial Communities), will be derived form good performing live food cultures, healthy farmed animals, and organically rich environment. The bacterial mixtures will be tested on live food (rotifers and *Artemia*) and fish eggs and larvae. The benefit for the seabream hatcheries sector will be, thanks to using RMBC's, the development of a sustainable, more predictable method of production of live food and larval fish and the exclusion of antibiotics.
Research team: CCMAR: Maria Teresa Dinis, Pavlos Makridis
Total budget for CCMAR: 50.000 Euros

Title: "Improving production efficiency of sea bass farming by developing methodologies to eliminate environmental androgenesis"
Summary and Objectives: The overall objective of the project is to understand the mechanisms regulating sex differentiation in cultured sea bass, in order to develop methodologies to minimize the proportion of males in cultured stocks.

Reference and funding entity: European Commission
Duration: 1/2001-12/2003
Research team: Coordinator: Silvia Zanuy, Consejo Superior de Investigaciones Cientificas – Spain; Costadinos Mylonas, IBMC, Greece; Francesc Piferrer, CSIC, Barcelona, Spain; Glen Sweeney, Univ. Wales; Abigale Elizur, Elat, Israel; UK; CCMAR: Adelino Canário, Rute Martins, João Condeça.
Total budget: 239.832 Euro; **Funding for CCMAR**: Euro

Title: "Feed for aquatic animals that contain cultivated marine microorganisms as alternatives for fish oil. **PUFAFeed** "
Reference and funding entity: European Community RTD Project QLRT- 1999-30271
Duration: Jan 2000 – Dez 2003
Summary and Objectives: The main objective of the current project is the development of alternative feed resources to fish meal and fish oil employing heterotrophic and mixotrophic microorganisms in order to supply the aquaculture industry with feed of constant and good quality that are free of toxins or genetically modified materials. In addition, feed based on a combination of heterotrophic and autotrophic produced microorganisms will be developed. PUFAfeed aims at the evaluation of Single Cell Oils (SCO), obtained from micro-organisms which include microalgae and diatoms, that are rich in the polyunsaturated fatty acids docosahexaenoic acid (DHA) and / or eicosopentaenoic acid (EPA) as alternative / complementary feed ingredients for fish oils. Within the project fundamental aspects of lipid accumulation in algae will be elucidated and the technologies to produce microbial biomass and novel feeds, based on this biomass, will be developed. Furthermore the performance of this feed and the economic feasibility of the integrated process will be established in order to provide a cost-effective alternative or complementary solution for fish oils use in feeds for aquaculture.
Research team: CCMAR: Maria Teresa Dinis, Florbela Soares, Pedro Cação
Total budget: 48.000 Euro; **Funding for CCMAR**: Euro

Title: "Fish restocking associated to the Algarve artificial reefs: environmental mitigation, biodiversity and fisheries management – **RESTOCKING**"
Summary and Objectives: The project objectives are related to: (i) evaluate the efficiency of restocking of finfish species associated with artificial reefs, using native species where the artificial reproduction and juvenile production are standard procedures, and (ii) develop the methodology necessary for the production in captivity of juveniles of other native with interest for restocking of artificial reefs, and whose populations are depleted as a result of a long and intense fishing effort.
Different methodologies will be developed in order to optimise tagging and releasing techniques. Initially, this will be done for available juveniles of *Sparus aurata* (gilt head seabream) and *Diplodus sargus* (white seabream). In a later stage, this will be done for juveniles of species which production methods will be developed during the project, (grouper *Epinephelus* sp. and the red porgy *Pagrus pagrus*). It should be noted that these are species with 1) ecology interest in relation to artificial reefs; (2) economical interest in terms of fisheries; and (3) interest for the revitalisation of over-exploited marine resources.The evaluation of restocking of fish in artificial reefs areas will be done in function of the size of the specimens, the season and the types of reefs. Classic methodologies will be used in this project, based on analysis of catch (recapture) and direct observation (underwater surveys) carried out in a regular basis. This restocking initiative will be advertised to the general public, in particular fishermen and anglers, to maximise the recapture records. Standard procedures for juvenile production in captivity of species that are not produced in Portugal and of interest for restocking (i.e., grouper and red porgy) will be developed. In particular, methodologies for induction of spawning in captivity and adequate feeding regimes and zootechnical conditions will be studied. This will be done using the methodologies available for other species as reference. This project expects to contribute for an integrated coastal management, promoting biodiversity, biological production and environmental mitigation. It will also contribute to the management of the local fisheries in order to promote their sustainability, with the

subsequent socio-economic effects.
Reference and funding entity: Fundação para a Ciência e Tecnologia, POCTI 35608/99
Duration: Jul 2001- Jun 2004
Research team: CCMAR: Maria Teresa Dinis, Luis Conceição, Marc Lacuisse, Florbela Soares; IPIMAR: Carlos Costa Monteiro, Miguel Neves dos Santos, Miguel Gaspar, Pedro Pousão-Ferreira.
Total budget: 149.639 Euro; **Funding for CCMAR**: 74.221 Euro

Division of Living Resources

New and Ongoing beyond 2005

"Recrutamento de Espécies Piscícolas de Interesse Comercial no Estuário do Rio Arade" (DGPA – MARE P.O. Pescas: 22-05-01-FDR-00017). Jorge Gonçalves.

Title: "**LOBASSESS** – Norway lobster stocks in Portugal. Basis for assessment using information on larval production and ecology"
Summary and Objectives: 1.- Estimate abundance and propose management options based on the understanding of larval recruitement processes.
2- To investigate larval eschanges among the different adult populations along the Portuguese coast.
Reference and funding entity: POCTI/BIA-BDE/59426/2004
Duration: 2 years
Research team: Margarida Castro (coordinator of the U. Algarve participation), Sara Mira.
Total budget: **Funding for CCMAR**:
Web site:

Title: "Fishery of the common octopus in Algarve: Improvement of the Artisanal fishing gears (POLVARTE)" (Ref: 22-05-01-FEDER-00018)
Summary and Objectives: Main objectives of this project are: 1) comparison of two of the most important fishing gears to catch octopus – pots and iron traps ("covos"), in relation to their catches; 2) introduction of a new type of plastic pot to catch octopus; 3) to study the behaviour of octopus towards pots (clay and plastic) and iron traps ("covos"); 4) to study possible environmental impacts of the use of plastic pots in comparison to clay pots; 5) study of the economic cost-benefit on the use of each of the octopus fishing gears.
Duration: 2003-2005
Funded by: Programme MARE, DGPA
Research team: Teresa Cerveira Borges (coordinator), Anxo Conde, Sónia Olim
Total budget: 105 053.15 € **Funding for CCMAR:** 105 053.15 €

Title "Biodiversity in fisheries off the South coast of Portugal (Algarve) (BIOFISH)" (Ref: 22-05-01-FEDER-00031)

Summary and Objectives: The main objective of this project is the compilation, characterization and photografic registration of all faunal species caught by commercial fisheries off the south coast of Portugal (Algarve), not only known commercial species, but also non-commercial species, which are generally discarded due to their inexistent commercial value in Portugal. All species caught by the most important fishing gears (trawl, purse seine, trammel net, longline, etc) will be identified, photographed, and all biological, ecological and fisheries information as well as their socio-economic importance, will be compiled. The result will be the production of a book with all species caught by fishing gears used off the coast of Algarve, to divulge in the fishing community (fishermen, researchers, managers) and public in general. A reference collection will also be criated, open to all community.
Duration: 2005-2007

Funded by: Programme MARE, DGPA
Research team: Teresa Cerveira Borges (coordinator), Sónia Olim, Paulo Morais, Luís Fonseca, Margarida Cristo, Margarida Machado, Jeff Wallace, João Sendão, José Xavier, António Malaquias, Carlos Afonso, Esmeralda Costa, João Gomes, David Francisco (fotógrafo).
Total budget: 246 052 € **Funding for CCMAR:** 246 052 €

Title: "**ALIENS** - Algal Introductions to European Shores"
Summary and Objectives: 1.- To explain the underlying ecological causes of the introduction, establishment and development of seaweed invasions on European shores
2.- To generate a baseline dataset on the present status of seaweed introductions to European shores, and of future susceptibility to further introductions/invasions
3.- To elucidate the genetic structure of various populations of selected invasive seaweeds in Atlantic and Mediterranean Europe, with a view to determining whether there have been multiple cryptic European introductions
4.- To evaluate the economic impact of existing seaweed invasions on a European scale, comparing losses with costs associated with prevention and eradication
5.- To carry out risk assessment and propose a screening protocol for invasive macroalgae to be used in coastal zone management
Reference and funding entity: EVK3-2001-0008
Duration: 3 years
Research team: Rui Santos (coordinator), Ester Serrão, Gareth Pearson, Aswin Engelen
Total budget: **Funding for CCMAR**: 177 000 Euro
Web site:

Title: "**CEPHSTOCK** -Cephalopod stocks in European waters: Review, Analysis, Assessment and Sustainable Management"
Summary and Objectives: This project aims to review current knowledge and issues in cephalopod fisheries science, to assemble, organise, analyse and synthesise data from ongoing national projects, previous and new EC-funded R&D projects, and to recommend future actions for scientists and cephalopod fishery managers in European waters. This will be achieved through (a) co-ordinated reviews and (b) development of common databases and associated GIS analysis capability, leading to (c) a series of co-ordination meetings and workshops at which the status of fished European cephalopod stocks will be reviewed, stock assessments carried out and management recommendations proposed.
Reference and funding entity: (QOL-2001-5.1.2) EC- DGXII
Duration: 01-10-2002 to 30-09-2005
Research team: University of Aberdeen (Coordinator) and 20 institutions from all Europe; from CCMAR/UAlg Teresa Cerveira Borges (coordinator) and Pedro Andrade.
Total budget: **Funding for CCMAR:** 27 600 Euro

Title: "Chloroplast gene expression in intertidal macroalgae (**CHLORGEN**)"
Funding institution: FCT (POCTI / 38863 / BSE / 2001.)
Duration: Sept 2002-Aug 2005
Objectives/Summary: The aim is to evaluate whether the variability in photosynthetic parameters and gene expression in *Fucus vesiculosus*, is related to circadian and/or circatidal signals and/or endogenous rhythms. The work involves determining gene sequences from macroalgal chloroplasts (plastids) to study their regulation and control of expression in photosynthetic organisms living in intertidal environments.
Research team: G Pearson, E Serrão, A Lago-Leston, M Valente
Funding: 61 632 Euro

Title: "**EPICAH** - Ecological and population impact of commercial agarophyte harvesting"

Summary and Objectives: - To assess the ecophysiological differences between life cycle phases that can explain the genetic structure of natural populations, *i.e.*, the relative abundance of the haploid and diploid life history phases.
- To assess the effect of the commercial harvest on the genetic structure of the populations and its demographic consequences;
– To develop structured population models, including both life history phases to simulate the observed harvest impacts.
- To assess the impact of the *G. sesquipedale* commercial harvest on the associated fauna of the fronds, and its consequent effects along the trophic web, with particular attention to the local commercial fish species.
Reference and funding entity: PDCT/P/Mar/15299/1999.
Duration: 2000-2004
Research team: R Santos, R. Carmona
Total budget: **Funding for CCMAR**: 120 000 Euro

Title: "Formosa dune: dinâmica espaço-temporal da vegetação de ilhas barreira da Ria Formosa"
Summary and Objectives: Evaluation of the ecological succession of vegetation of barrier islands in the Natural Park of Ria Formosa.
Reference and funding entity:
Duration: 2 years
Research team: R Santos (CCMAR) J. Fernandes
Total budget: 35000 Euro; **Funding for CCMAR**: 35000 Euro

Title: "Global related changes in the Portuguese marine flora over a long time scale"
Summary and Objectives: The main aim of this proposal is to describe the long-term changes in the benthic marine flora of the continental coast of Portugal by comparing the actual situation with the only available description of the Portuguese marine flora, which was done in the 1960´s by Ardré (1970, 1971).
Reference and funding entity: POCTI/BSE/48918/2002
Duration: 2003-2006
Research team: R Santos. E. Barecibar, J. Silva
Total budget: **Funding for CCMAR**: 92 000 Euro

Title: "Instrumentos promotores de formação e de participação dos cidadãos: EqEA - Equipamentos para a Educação Ambiental em Portugal"
Summary and Objectives: We aim to assess the environmental equipments in Portugal, their implementation and development. Criteria will be established to evaluate them.
Reference and funding entity: Protocolo de colaboração entre o Instituto do Ambiente e o CCMAR
Duration: 2003-2005
Research team: R Santos. H. Barracosa
Total budget: **Funding for CCMAR**:

Title: "Monitoring and Management of European Seagrass Beds (**M&MS**)".
Summary and Objectives: To evaluate the status of European seagrass beds, particularly for CCMAR to evaluate their capacity for recovery via sexual reproduction and their genetic diversity and structure
Funding institution: EU- EnvSD (EVK3-CT-2000-00044)
Duration: Feb 2001-Aug 2005
Research team: At CCMAR-UAlg: E. Serrão, S. Arnaud-Haond, F. Alberto, O. Diekmann, M. Billingham, R. Santos, S. Cabaço, R. Machás, A. Cunha, *et al*.
Funding for CCMAR: 330 000 Euro

Title:"Recruitment of Fish Species of Commercial Interest in the Arade River Estuary"

Summary and Objectives: Knowledge concerning estuarine fish communities is particularly important for the sustainable management and exploitation of our natural resources. Given that the estuary of the Arade river is one of the largest in the south of Portugal, there is a pressing need to better understand the ichthyofauna and how the different fish species use the estuary. Information concerning the type of recruitment, migrations and habitat use (spawning, shelter and feeding), especially with regards to juveniles of commercially important species, is of primary importance for coastal fishing activity and the conservation of these resources. On the other hand the existence of protected or threatened species and habitats should be evaluated in a way that sustainability can be ensured by means of appropriate measures. The dissemination of this knowledge is urgent given the utility for the public in general and for the various public and private economic entities involved. It is not enough to merely know it is also necessary to inform so that a sustainable relationship between man and the natural resources can be established and perpetuated. The objectives of this project are twofold: 1- The characterisation of the structure of the fish community and the distribution of the different species in the estuary of the Arade river, with particular emphasis on the juvenile stages of commercial and threatened species and the interactions between their distribution and abundance and estuarine environmental parameters. 2 - The dissemination of scientific knowledge to the public in general, to public and private entities and to the scientific community.
Reference and funding entity: MARE Programme - P.O. Pescas (Cód. 22-05-01-FDR-00017)
Duration: 2003-2006
Research team: CCMAR: Jorge M.S. Gonçalves and Karim Erzini
Total budget: 139170 Euro; **Funding for CCMAR**: 139170 Euro
Web site: http://www.ualg.pt/fcma/cfrg/

Title: "Signals for gamete release by intertidal species with external fertilization (**GAMETE**)"
Funding institution: FCT (POCTI / 39431 / BSE / 2001.)
Duration: Mar 2002-Feb 2005
Objectives/Summary: Study of factors that affect the reproductive success of externally fertilizing fucoid algae, such as signals for synchronous gamete release, timing for gamete release during tidal cycles and lunar cycles, consequences for dispersal and recruitment.
Research team: E Serrão, G Pearson, C. Faustino, C. Monteiro.
Funding: 45 000 Euro

Title: Nutritional condition of fish larvae in two marine protected area of the South of Portugal (Ria Formosa and Guadiana estuary) **(Guadiria)**
Summary and Objectives: In the south of Portugal there are two important and contrasted marine protected areas: the Ria Formosa and the Guadiana estuary. The Ria Formosa is a tidal coastal lagoon, with low depth and high rate of water exchange with the sea, and the Guadiana river has is a medium estuarine area, with an irregular hydrological regime, with severe droughts and floods, and its under increasing pressure for exploitation of water resources, being the construction of Alqueva dam the most recent example. Both systems are highly productive and provide ideal conditions for initial phases of fish's life cycle.
Besides previous studies, that have show that these coastal systems are important nurseries areas for juveniles, especially the salt-marshes areas, for many commercially important fish species, several issues are still not understood. They are: Is the nutritional condition of fish larvae higher inside these systems compared with the same species captured in coastal zone? Is this due to higher food availability or to higher predation inside the systems that remove weak larvae quickly or to absence or retention strategies? Are there, from typical fish species, some more sensitive to inanition then others? Are larval species from benthonic eggs more resistant to adverse environmental condition than that from pelagic egg? Our main objective will be an in depth investigation of the nutritional condition of larval phase of the fishes inside the Ria Formosa and the Guadiana estuary. Specific objections are the answers to the above questions, through the use

of nucleic acids derived indices, such as RNA/DNA, RNA residuals and DNA per mg of larva dry weight. Those indices have been used successfully in several larval fish species in different phase of its development to access their nutritional condition. This project will tstudy the relative importance of ontogenic (benthonic or pelagic eggs) or ecohydrologic factors (currents, winds, food availability) in the nutritional condition of fish larvae in the selected systems. It will be selected fish species residents in these systems such as Gobidae with benthonic eggs and larvae from planktonic eggs, usually temporary species, such as Soleidae or Sparidae for the Ria Formosa, and Engraulis encrasicolus for the Guadiana estuary. The sampling of this larval species will be done inside the Ria Formosa and the Guadiana estuary and in both adjacent coastal areas. Light trap will be use to capture the fish larvae, in order to minimize the physiologic stress cause by net tow and to increase the size of fish larvae caught by the traditional icthyoplanktonic gears. The results of the present study obtained in different marine protected coastal areas, will help in the support or refute an important hypothesis in larval ecology and recruitment field, “the member vagrant or retention hypothesis”, that link high larval condition (and future recruitment) with successful strategies of retention during the larval stage in adequate areas.
Reference and funding entity: POCTI/BIA-BDE/59200/2004 , FCT
Duration: Dez 2004-Dez 2004
Research team: CCMAR: Maria Alexandra Chícharo, Luis Chícharo, Ana Amaral
Total budget: 42620 Euro; **Funding for CCMAR**: 42620Euro

Completed in 2004

Title: "Avaliação experimental do impacte nas biocenoses marinhas associado à exploração de manchas de empréstimo ao largo do Algarve Central” (DRAOTA-RENSUB).
Summary and Objectives: The impacts of dredging on the aquatic environment are generally considered to be temporary, short term, high intensity perturbations that can result in temporal changes in the populations. Nevertheless, there are in general few in depth studies concerning the type and evolution of the changes in populations affected by the extraction of sand and gravel from the marine sub-tidal zone, with none from Portugal. Thus, the study of the impacts on biological communities of dredging for the purposes of beach nourishment is in certain aspects pioneering at the national level and may be of considerable use as a reference for future projects in similar areas. The objective of this project is to characterise, in baseline terms, the biological component of the underwater zone between Albufeira and Vale do Lobo-Quarteira (Central Algarve) as well as to evaluate the impacts resulting from the extraction of sand and gravel by means of systematic monitoring of the impacted marine communities.
Reference and funding entity: DRAOTA Protocol
Duration: 2002-2004
Research team: CCMAR: Jorge M.S. Gonçalves, Karim Erzini.
Total budget: € 85702; **Funding for CCMAR**: €85702
Web site: http://www.ualg.pt/fcma/cfrg/eng/projects/rensub.shtml

Title: “Experimental By-catch Reducing Devices (BRD) in the demersal purse-seine fishery and evaluation of survivorship”
Summary and Objectives: Preliminary studies have shown that mean discard rates in Algarve (southern Portugal) purse seine fisheries vary between 0.20 and 0.30; with between 5,000 and 10,000 mt of discards per year (Borges et al., 1997, 2000, Erzini et al., 2001). The objective of the proposed project is to test simple by-catch reducing devices (BRDs) for the demersal purse seine in order to reduce the by-catch and discarding of under-sized and/or non-commercial species. In particular, we will evaluate the use of both larger mesh sizes and square meshes in part of the net. These modifications should increase the escapement of juveniles and non-commercial species. Quantification of the effectiveness of BRDs will be carried out by means of experimental fishing trials using small-mesh covers over the BRD sections. In addition, the condition of fish that have escaped will be evaluated and post-escapement survivorship monitored in tanks. The relationships between condition (scale loss and other signs of external damage), stress (monitored by cortisol

radioimmunoassay) and long-term survivorship will be evaluated experimentally. The results of these experiments will be transmitted to the fishing community.
Reference and funding entity: FCT/POCTI/BSE/43113/2001
Duration: 2002-2004
Research team: CCMAR: Jorge M.S. Gonçalves, Karim Erzini and Adelino Canário
Total budget: 73000 Euro; **Funding for CCMAR**: 73000 Euro
Web site: http://www.ualg.pt/fcma/cfrg/

Title: Fishing impacts. Use of control areas to assess fishing impacts of dredge activity_
Aims: To anlyse the dredge fishing impacts through the use of control areas to assess fishing impacts on the south cost of Portugal
Duration: 01-012-2002 a 01-012-2004
Reference and funding entity: FCT Programa SAPIENS (POCTI/MGS/42319/2001)
Research team: Luís Chícharo (**Responsável na Univ. Algarve**), Alexandra Chícharo (Universidade do Algarve), Carlos Costa Monteiro (IPIMAR CRIP/Sul); Miguel Gaspar (Coordenador, IPIMAR CRIP/Sul).
Total budget: 30.247,5 EURO **Funding for CCMAR**: 0

Title: "Ecological and socioeconomic impacts of river inflow changes in three Portuguese rivers on the estuaries and coastal areas**"**
Aims: To analyse the ecological and socioeconomic impacts of river inflow changes in three Portuguese rivers (Guadiana, Tagus and Minho) on the estuaries and coastal areas
Duration*:* 01-01-2000 a 01-01-2004
Funding: FCT Programa FCT/MAR/15263/99
Team: Maria José Costa (Instituto de Oceanografia da Faculdade de Ciências (Coordenadora), Alexandra Chícharo (Responsável na Univ. Algarve), Luís Chícharo (Universidade do Algarve)
Budget: *24000 EURO* **Funding for *CCMAR:*** 0

Title: "Local adaptation and population genetic structure in intertidal algae" (GENFUCUS)
Funding institution: FCT (POCTI/BSE/35045/99)
Duration: Dec 2001-Nov 2004
Objectes/Summary: Populations of two closely related algal species with contrasting fragmented distributions are investigated concerning their population genetic structure and divergence.
Research team: CCMAR-UAlg: E Serrão, G Pearson, C Daguin, C Engel, *et al*.
Funding: 150 000 Euro

Title: "The Portuguese coast as a biogeographic boundary: consequences for reproductive success, local adaptation and genetic structure of populations living at their distributional limits (**BIOPORT**)"
Funding institution: FCT (PDCTM / P / MAR / 5292 / 1999)
Duration: Feb 2001-Jan 2004
Objectes/Summary: To compare the reproductive success and ecophysiological differentiation of species at their southern distributional limits, and their genetic consequences.
Research team: CCMAR-UAlg: E Serrão, G Pearson, C Daguin, L Ladah, C Engel, et al.
Funding: 225 000 Euro

Title: "**SEMAPP** - Science, Education and Marine Archaelogy Programme in Portugal "
Summary and Objectives: Co-operation to study the marine ecosystem at selected locations in Portuguese territorial waters. The aim of this programme is to develop the knowledge and research of oceanography related to fisheries, an important and desirable area of research in Portugal. The University of Connecticut has access to, and expertise in utilising underwater systems and technologies that allow for detailed *in-situ* observations and research in water depths up to 2,000 meters. The initial proposed study site is Portimão Submarine Canyon and adjacent

Continental Slope and Shelf region, off Portugal's Algarve coast. The plan is to spend the first 2 years conducting detailed bathymetric surveys and sub bottom profiling in order to map the ocean floor in those regions. In 2004, it is planned using manned submersibles and robot technology to assist with studies in Fisheries Biology, Benthic Ecology and Environmental Sciences.
Duration: 2000 …
Funded by: OTF, USA & others
Research team: University of Connecticut: Prof. Richard Cooper (co-ordinator of programme); CCMAR: Teresa Cerveira Borges (Marine Biology); Autonomous University: Dr. Adolfo Silveira Martins (Marine Archaeology); Hidrographic Institute.
Total budget: variable **Funding for CCMAR:**

Title: "**SEAPURA**. Species diversification and improvement of aquatic production in seaweeds purifying effluents from integrated fish farms"
Summary and Objectives: Use of seaweeds of economic value to biofilter fish farm effluents.
Reference and funding entity: QLRT - 1999 - 31334
Duration: 2001-2004
Research team: R Santos. L. Mata and A. Schuenhoff
Total budget: **Funding for CCMAR**: 150 000 Euro
Web site: http://www5.ulpgc.es/servidores/algologia/seapura.html

Title: "Monitorização de lontras costeiras no Sudoestes de Portugal [Monitoring coastal otters in southern Portugal]"
Summary and Objectives: This project aims at monitoring the population of otters inhabiting the coastline of southwestern Portugal. For this, the project will use DNA markers recovered from faeces to identify individual otters occurring along the coast. This will allow for an estimate of population numbers and habitat preferences. The project will also optimize techniques for the extraction and analysis of DNA in otter faeces, with potential applications to other species of endangered mammals.
Reference and funding entity: Transgás Atlântico, SA
Duration: January 2001 –December 2004
Research team: Pedro Beja, Leonor Cancela, Sara Mira, Catarina Canas
Total budget: 47386 Euro; **Funding for CCMAR**: 47386 Euro

Title:"Resilience and genetic diversity of seagrasses affected by anthropogenic perturbations in the Natural Park of Ria Formosa (**SEAGRASSRIA**) "
Funding institution: FCT (PNAT/1999/BIA/15003/C)
Duration: Apr 2001-Mar 2004.
Objectives/Summary: Evaluation of the genetic structure and reproductive ability of the seagrasses in the Natural Park of Ria Formosa.
Research team: CCMAR-UAlg: E Serrão, R Santos, M Billingham, F Alberto, *et al*.
Funding: 75 000 Euro

Title: "Scientific bases for the management of fisheries resources of common interest (Bases científicas para a gestão de recursos pesqueiros de interesse comum)"
Summary and Objectives: This INTERREG III project, co-ordinated by the Centro Regional de Investigação Pesqueira do Sul of IPIMAR, involves the CFRG (CCMAR), and the Centros de Investigación y Cultivo de Especies Marinas de Agua del Pino (Huelva) y "El Toruño" (Cádiz) of the Consejería de Agricultura y Pesca (Junta de Andalucía, Spain). The CFRG is responsible for studies focusing on understanding the spatial and temporal scale of fish movements and habitat use, with emphasis on several species of sea breams (Diplodus sargus, Sparus aurata) and the artificial reefs located on the Algarve continental shelf. Long-term automated monitoring of fish tagged with pingers will be carried out using Ultrasonic Receiver/monitors fixed on the bottom. Short-term (daily movements) will be studied by tracking tagged fish from a small vessel with a directional hydrophone and ultrasonic receiver. The acoustic telemetry data will provide important

information on the spatial and temporal dynamics of these species. In particular, it will be possible to obtain data on habitat preferences, habitat use, daily activity patterns, home ranges and long-term movement patterns. The results of this project will contribute to better conservation and management of the fisheries of the Algarve, where sea breams and bass are an important component of the demersal fish community and of the fisheries landings and where artificial reefs and stock enhancement (sea breams and other species) are important elements of the longterm management strategy.
Reference and funding entity: INTERREG III (Projecto GESTPESCA)
Duration: 2002-2004
Research team: CCMAR: Karim Erzini and Jorge M.S. Gonçalves, IPIMAR and Junta de Andalucia
Total budget: 52500 Euro; **Funding for CCMAR**: 612100 Euro
Web site: http://www.ualg.pt/fcma/cfrg/

International and Inter-Institutional Cooperations

Division of Aquaculture and Biotechnology

Treaty of Windsor 2004/2005 Com a Universidade de Bath, UK. Coordeandores: L. Cancela, (Portugal) e Robert Kelsh (UK). Participantes: Paulo gavaia e Brigite Simões.

Title: "Identification of Sex Pheromones from the Anal Gland of Male Blennies, *Salaria pavo* and *S. fluviatilis* (Pisces: Blenniidae)."
Summary and Objectives: *Salaria pavo* is small bottom living fish in the shallow littoral zone of the Mediterranean and adjacent Atlantic coast. The closely related freshwater species, *S. fluviatilis*, inhabits rivers and lakes in the vicinity of the Mediterranean. In both species, the mating system is promiscuous. Males occupy holes or crevices in the hard substrate where females come to spawn and the males subsequently guard the eggs. These are a good model fish species to investigate specialization in sex pheromone production. The males develop anal glands from the epidermis of the first two rays of the anal fin concurrent with development of the gonads. The Portuguese team has shown that the anal gland of *S. pavo* is a source of substances that attract pre-ovulatory females which promotes male reproductive success. This suggests that male blennies are "active signallers" in contrast with known pheromone systems in teleosts, where receivers are "chemical spies" detecting gonadal steroids or prostaglandins passively excreted by females into the water *via* the urine or gills.
The proposed work aims to identify the chemical structures of putative sex pheromones in the two species. Although strictly a freshwater species, the marine origin of *S. fluviatilis* is well established and, as with *S. pavo*, it is plausible that the anal gland has a pheromonal function in female attraction. If so, one would expect the pheromonal components of the two species to have similar or closely related chemical structures. In addition, the inclusion of *S. fluviatilis* in the project will simplify the chemical identification, since recording of electro-olfactograms is technically easier in freshwater, and can be combined with chromatographic separation of anal gland-produced substances.
Reference and funding entity: British Council/CRUP (Acções Integradas Luso-Britânicas nº B-14/03)
Duration: January 2003 - December 2004
Research team: CCMAR: Eduardo N. Barata, Peter C. Hubbard, Ana Freitas, Rui Serrano, Adelino V.M. Canário. Rothamsted Research – Biological & Ecological Chemistry Division, UK: Mike Birkett, Lester Wadhams & John Pickett.
Total budget: 6.000 Euro; **Funding for CCMAR**: 3000 Euro

Title: Identification of food-related attractants for the Senegal sole, *Solea senegalensis.*

Summary and Objectives: Fishes rely upon information received by all their senses for food detection, recognition and selection. Feeding behaviour shows a stereotyped sequence of behavioural components, including arousal, searching, food uptake and ingestion. The arousal phase is mostly mediated by olfaction, whereas in the searching phase the relative importance of different sensory modalities varies depending upon the feeding strategy and ecological niche of the species. Feeding behaviour is completed by food uptake and ingestion phase, which is triggered by chemical stimuli.
In different species, different chemical substances stimulate feeding behaviour. Some of these may act as attractants via olfaction, and others may act as promoters or enhancers of food consumption via both olfaction and gustation. Amino acids, nucleotides, nucleosides and quaternary ammonium bases have been identified as feeding stimulants from experiments with juveniles of different species. For example, glycil betaine, trimethylglycine and dimethylthetin have been reported as feeding stimulants for juvenile Dover sole, *Solea solea*. Some species specificity for feeding stimulants seems to exist, and in general mixtures are more effective than single compounds.
The Senegal sole (*Solea senegalensis*) is a good model species to investigate olfactory mechanisms underlying feeding behaviour, due to its feeding strategy, and a well-developed olfactory system accessible to electrophysiological recordings. The central aim of this project is to identify substances released by natural food sources (polychaets) that act as olfactory cues involved in food-search behaviour. This objective will be achieved by an integration of electrophysiological and behavioural measurements in response to candidate olfactory stimuli. Ultimately, the project will identify the chemical cues that are important in the initiation of food-search behaviour. This may be the ground for future technological development of new inert food diets that enhance the farming of this commercially important species.
Reference and funding entity: British Council/CRUP (Acções Integradas Luso-Britânicas nº B-71/04)
Duration: January 2004 - December 2004
Research team: CCMAR: Eduardo N. Barata, Peter C. Hubbard, Zélia Velez, Adelino V.M. Canário. University of Hull – Department of Biological Sciences, UK: Thomas Breithaupt, Helga Bartels-Hardege, Ralf Bublitz, Victor Sweetez & Joerg Detlef Hardege.
Total budget: 2.900 Euro; **Funding for CCMAR**: 1.450 Euro

Title: "Ontogeny of the endocrine system in *Solea senegalensis* and its application to the improved rearing of larvae"
Summary and Objectives: The investigation of new species for aquaculture, such as the sole (*Solea senegalensis*) contributes significantly to the development of marine fish culture in Spain and Portugal. However, one major bottleneck to the expansion of the industry is the rearing of larval fish. The industry relies on the production of a large number of high quality fry, which in turn relies on the successful first feeding, development and growth of the fish larvae. Embryonic development of marine fish is rapid and larvae hatch at a relatively early stage of development, resulting in an increased vulnerability to external factors. As hormones play an important role in regulating feeding, growth and metamorphosis, it is important to examine the structural and functional development of the endocrine system during early ontogeny of marine fish. This project aims to 1) investigate the development of the endocrine system in *Solea senegalensis* 2) determine how fish larvae respond to stressful situations and 3) study the possible beneficial effects of exogenously added hormones on growth, metamorphosis and pigmentation.
Reference and funding entity: CRUP (Acções Integradas Luso-Espanholas)
Duration: Jan 2004 – Dec 2005
Research team: CCMAR: Maria Teresa Dinis, Neil Ruane; Instituto de Ciencias Marinas de Andalucia, Spain - Maria del Carmen Sarasquete.
Total budget: 2.200 €; **Funding for CCMAR**: 2.200 €
Web site:

Title: "Histomorphology, citohistology and ultrastructural study of *Solea senegalensis* pigmentation under different rearing conditions"
Summary and Objectives: The main objective of the project is to optimise larval cultivation of sole *(Solea senegalensis*), a species with high potential for marine fish farming. As a consequence of an ongoing project some bottlenecks for sole cultivation were identified. These included control of reproduction, weaning and pigmentation abnormalities (total or partial albinism). This project focuses on this last problem. A significant proportion of pigmentation abnormalities may occur in hatchery produced sole, and are a problem for marketing of this species. Pigmentation abnormalities in sole larvae and post-larvae will be studied using histochemical and immunohistochemical techniques. It will be investigated whether zootechnical parameters (density, temperature, salinity, photoperiod, light intensity, type of substract) or nutritional factors are related to pigmentation abnormalities.
Reference and funding entity: Convénio ICCTI/ CSIC
Duration: Dez 2000-Dez 2004
Research team: CCMAR: Maria Teresa Dinis, Florbela Soares, Laura Ribeiro, Pedro Cação; Instituto de Ciencias Marinas de Andalucia, Spain - Maria del Carmen Sarasquete, Emílio Pascual, Manolo Yúfera.
Total budget: 3.500 Euro; **Funding for CCMAR**: Euro

Title: Marine fish sperm cryopreservation

Summary and Objectives: Cryopreservation plays an important role in sperm cryopreservation for aquaculture. During the last two decades sperm cryopreservation helped to solve problems related with fish reproductive dysfunction, such as asynchronous maturation of breeders or problems due to captivity stress, facilitating the planning of crossbreeding. This technology has been specially developed for salmonid species, and at the moment, several fish farms around the world take advantage of sperm cryopreservation in the management of their broodstocks. The marine species, gilthead seabream (*Sparus aurata*) and Senegal sole (*Solea senegalensis*) are two good candidates for the application of cryopreservation procedures. This technique would also allow the maintenance of a high quality sperm stock that could be used for different purposes.

The aim of the present project is to develop a simple and reliable test to assay sperm quality during the spawning period as well as to develop a cryopreservation protocol for each species to be applied in commercial scale conditions. Both teams will work together in the sperm cryopreservation procedures. Their different experiences and technical support will allow for the development of new protocols and cryopreservation procedures specifically design for this two high value marine species.

Reference and funding entity: Ministério da Ciência e do Ensino Superior- Acções bilaterais, Grices/CSIC Proc. 4.1.1/CSIC

Duration: Jan 2004-Dec 2005

Research team: CCMAR: Jeff Wallace, Elsa Cabrita, Florbela Soares; ICMAN-CSIC: Maria del Carmen Sarasquete, Juana Arellano

Total budget: 1.396 euros

Division of Living Resources

Mercado, Jesús - Instituto Español de Oceanografía, Málaga. Determinación del índice $\delta^{13}C$ y su relación con la actividad da anidrasa carbónica en productores primarios de la costa sur de la Península Ibérica Acordo de Cooperação GRICES/CSIC (contact person in CCMAR: Rui Santos)

Duarte, Carlos - IMEDEA, Islas Baleares. DOM production from contrasting communities in the Ria Formosa (S. Portugal), and export to the Atlantic Sea. Acordo de Cooperação ICCTI/CSIC (contact person in CCMAR: Rui Santos).

Title: "**SEMAPP** - Science, Education and Marine Archaelogy Programme in Portugal "
Summary and Objectives: Co-operation to study the marine ecosystem at selected locations in Portuguese territorial waters. The aim of this programme is to develop the knowledge and research of oceanography related to fisheries, an important and desirable area of research in Portugal. The University of Connecticut has access to, and expertise in utilising underwater systems and technologies that allow for detailed *in-situ* observations and research in water depths up to 2,000 meters. The initial proposed study site is Portimão Submarine Canyon and adjacent Continental Slope and Shelf region, off Portugal's Algarve coast. The plan is to spend the first 2 years conducting detailed bathymetric surveys and sub bottom profiling in order to map the ocean floor in those regions. In 2004, it is planned using manned submersibles and robot technology to assist with studies in Fisheries Biology, Benthic Ecology and Environmental Sciences.
Duration: 2000 …
Funded by: OTF, USA & others
Research team: University of Connecticut: Prof. Richard Cooper (co-ordinator of programme); CCMAR: Teresa Cerveira Borges (Marine Biology); Autonomous University: Dr. Adolfo Silveira Martins (Marine Archaeology); Hidrographic Institute.
Total budget: variable **Funding for CCMAR:** (150 000€, in 2004)

Rafael Zardoya, Departamento de Biodiversidad y Biologia Evolutiva, Museo Nacional de Ciencias Naturales, Madrid, Spain. Acordo de Cooperação ICCTI/CSIC (contact person in CCMAR: Rita Castilho).

CCMAR Seminar programme

List seminars

Seminars given by CCMAR members in other institutions

2004. Comunicação oral no Symposium "Towards an Integrated Knowledge for the Management of Estuarine Systems" 2004 Lisbon IO-ECSA Local Meeting, Instituto de Oceanografia, Faculdade de Ciências da Universidade de Lisboa em 9-10 de Setembro, sob o titulo “Fishes assemblages associated with shallow seagrass and bare sand in the Ria Formosa lagoon (Algarve, Portugal): seasonal, tidal and diel variations” e com os seguintes autores: Ribeiro, J, L. Bentes, J.M.S. Gonçalves, P. Lino, P. Monteiro, R. Coelho, K. Erzini.

Chícharo, L.- “Ecology of coastal ecosystems” and “Coastal and estuarine ecohydrology: the Guadiana case” – open seminar in the Ecohydrology master course in the University of La Plata, La Plata, Argentina. CCMAR Support: Latin-America Regional Office of UNESCO

Conceição, L.E.C. (Oct 2004). Aspectos Fisiológicos Básicos y Aplicados en Alimentación y Nutrición de Larvas y Juveniles de Peces. IX Curso Avances en Acuicultura y Calidad Ambiental”, Instituto de Ciencias Marinas de Andalucía (CSIC) y Facultad de Ciencias del Mar da Universidad de Cadiz, Puerto Real, Cadiz, Spain.

Conceição, L.E.C. (Feb 2004). Aquaculture of Senegalese Sole : Potential and Challenges. National Center for Mariculture, Israel Oceanographic and Limnological Research, Eilat, Israel.

Conceição, L.E.C. (Mar 2004). Avances en la alimentación y nutrición del lenguado. I Jornada Científico-Técnica Andalucía-Marruecos Diversificación de Especies en Acuicultura: Aspectos básicos y aplicados. 4-5 March 2004, Cádiz, Spain.

Erzini, K. “La selectividad de las artes de pesca en las pesquerias multi-específicas del Algarve”. I Conferencia Internacional sobre la Investigación Pesquera y Aquícola en el ámbito ibérico-marroquí. Cartaya (Huelva), 23 – 26 of November, 2004

Erzini, K. “Pescar e conservar, o desafio do futuro”. 2º Encontro Regional de Educação Ambiental do Algarve. Grande Auditório de Gambelas, Faro, 23 e 24 de Setembro de 2004.

Erzini, K. and Sá, R. “Guadiana food web”. “Managing the Guadiana estuary – the Ecohydrology and Phytotechnology approaches”. Workshop (in the framework of UNESCO IHP-VI), Faro, 1-2 Setembro.

Gonçalves, J.M.S.; Bentes, L.; Monteiro, P.; Coelho, R.; Ribeiro, J.; Lino, P. & Erzini, K.. Palestra intitulada “Pesca Fantasma” proferida na Expomar em Olhão a 21 de Março 2004.

Ribeiro, J.; Erzini, K.; Bentes, L.; Monteiro, P.; Coelho, R.; Afonso, C. & Jorge M.S Gonçalves. Palestra intitulada “Mergulho Cientifico” proferida na Expomar em Olhão a 19 de Março 2004.

Soares, F. (March 2004) . Patologias associadas aos cultivos em tanques de terra em Portugal. Jornadas de aquicultura. 24-25 March Cartaya, Spain.

Dissemination of scientific culture

Afonso-Dias, I. “A vida microscópica num tanque de água doce”. Actividade desenvolvida na escola EB1 de Alto Rodes de Faro, no âmbito da semana da Ciência em Novembro de 2004.

Cancela ML.. Participação na entrevista subordinada ao tema: “A ultima fronteira” . Expresso 20 Novembro 2004., p47.

Cancela, M.L. “Terapia Génica”. Seminário de divulgação na Escola Secundária de Albufeira. 2004

Cancela, ML. Journal “Algarve Académico”.Entrevista sobre Investigação desenvolvida pelo grupo liderado por L. Cancela no âmbito do CCMAR. Dezembro 2004

Cancela, ML. Seminários de alunos finalistas das licenciaturas em Biologia Marinha e Pescas e em Bioquímica, no âmbito da disciplina de Biotecnologia leccionada por L. Cancela. Escola Secundária Laura Ayres, Quarteira, Dezembro 2004

Conceição, L.E.C. Orientação de estágio integrado no programa Ocupação Científica de Jovens nas férias, promovido pela Agência Ciência Viva, dos alunos do ensino secundário Nehuen Crespi e Tiago José Viegas Ventura, com o tema “Cultivo de Peixes Marinhos”, no Centro de Ciências do Mar do Algarve (CCMAR), de 29 de Julho 12 de Agosto de 2004.

Gonçalves, J.M.S., Associação Atlântico Selvagem. 2004. Participação na exposição sobre as Expedições da AAS aos montes submarinos do Atlântico Norte, que decorreu no Centro Cultural de Tavira (antigo mercado municipal) de 15 Maio a 15 de Junho.

Gonçalves, J.M.S., Monteiro, P., Coelho, R., Afonso, C., Ribeiro, J., Almeida, C., Ramires, T., Veiga, P., Machado, D., Oliveira, F. e Bentes, L. 2004. Coordenação e concepção de Exposição REN Submarina, que decorreu na sede da CCDR Algarve entre 6 de Dezembro de 2004 e 6 de Janeiro de 2005.

Gonçalves, J.M.S.; K. Erzini; L. Bentes; R. Coelho; P. Monteiro; J. Araújo; M. Corado e I. Costa. 2004. Projecto BRD. Reduzir a pesca acessória na arte de cerco. Folheto de divulgação Nº8 Centro de Ciências do Mar (CCMAR), Universidade do Algarve. Autores:

MacMullen, P.; Hareide, N.-R.; Furevik, D.; Larsson, P.-O.; Tschernij, V.; Dunlin, G.; Revill, A.; Pawson, M.; Puente, E.; Uriarte, A.; Sancho, G.; Santos, M.N.; Gaspar, M.; Erzini, K.; Lino, P.; Ribeiro, J.; Sacchi, J. 2004. "Ghost fishing in European Waters". Video produced based on the FANTARED II project: A study to identify, quantify and ameliorate the impacts of static gear lost at sea. Final Report to the Commission of the European Communities, EU Study Contract FAIR CT98-4338. 490 pp + appendices.

Mira, Sara and ML Cancela. " Lontras debaixo de olho" Revista Focus 261/ 13 Outubro 2004, pp 50-52

Soares, F. Orientação de dois estágios de formação Profissional integrados no curso de técnico de Aquacultura do Forpescas, dos alunos Isadora Serro e André Simplício, no Centro de Ciências do Mar do Algarve (CCMAR), de 20 de Maio 12 de Agosto de 2004.

Visiting scientists

Abdelhafid Chalabi, lecturer at the Faculté des Sciences Biologiques, FSB / USTHB, Algeria visited CCMAR twice for periods of two weeks. During his stays K. Erzini worked with him on the modelling of bluefin tuna catch data with GLM and GAMs.

Carlos Duarte, IMEDEA, CSIC,Spain – supervision of PhD student Filipe Alberto, postdoctoral fellow Sophie Arnaud, and joint projects.

Dr. José Hernando Casal, University of Cádiz, was also a participant in the above mentioned trammel net project and visited for one week in November 2004 to discuss the special issue and the contents of the various manuscripts in preparation.

Dr. Kostantinos Stergiou, Aristotle Univeristy, Thessaloniki, Greece. Six month sabbatical from September 2004 – February 2005. During this period Dr. Stergiou collaborated in the preparation of a special volume of the journal Fisheries Research dedicated to trammel nets. The publications are the result of an international project on trammel net fisheries in Portugal, Spain and Greece.

Eric Wolskanki from the Australian Marine Institute. He developed an ecohydrology model to the low flow conditions in the Guadiana Estuary

Ivar Babb, National Undersea Research Center, University of Connecticut (guest of Teresa Cerveira Borges)

Mahfoudh Ould Taleb, researcher at the Institut Mauritanien de Recherches Océanographiques et des Pêches, Nouadhibou, Mauritania was helped in the analysis of trawl discard data.

Mar Huertas, IRTA, Tarragona, Spain. Investigations into the potential role of chemical communication in the reproduction of the eel (Anguilla anguilla).

Marouene Bdioui, researcher and doctoral candidate from the Institut National des Sciences et Technologies de la Mer «INSTM», Tunisia, spent two weeks in January at CCMAR where he was supervised in the estimation of size selectivity parameters for trammel nets used to catch the shrimp Penaeus kerathurus in the Gulf of Gabès.

Nuria Marba, IMEDEA, CSIC, Spain - supervision of postdoctoral fellow Elena Varela and joint projects.

Raúl Cruz – Universidade de Havana, Cuba. Setting of dacapod larvae collectors alons the Southweast coast of Portugal. (contact person in CCMAR: Margarida Castro)

Richard A. Cooper University of Connecticut (guest of Teresa Cerveira Borges)

Organization of Conferences, Workshops, Courses

"Introdução ao ArcGIS – Nível I" leccionado na Universidade do Algarve pela Dra. Rita Crespo, através de protocolo com a ESRI Portugal e certificado pela ESRI (Environmental Systems Research Institute). Realizado em Maio e com a duração de dois dias, este curso introduziu a tecnologia ArcGIS, a novos utilizadores de ArcView 8.x, ArcInfo 8.x e ArcEditor, incluindo a apresentação de conceitos GIS e a utilização prática das aplicações ArcMap, ArcCatalog e ArcToolbox.

Congresso Nacional de Bioquimica 2004, Vilamoura 4-6 Dezembro. Presidente da Comissão organizadora: ML Cancela. (~700 participants)

Course: Analysing Biological and Environmental Field Data, 9-13 February, 2004, Instructor: Dr. Alain Zuur, Highland Statistics Ltd.

Course: Introduction to ArcGIS – Level II., May 2004 (3 days). Instructor: Dr. Rita Crespo, through an agreement with ESRI Portugal and certified by ESRI (Environmental Systems Research Institute). este curso destinou-se à especialização em ArcGIS, focalizando-se na análise espacial, automação de

dados espaciais e de dados alfanuméricos, edição e opções avançadas de visualização e produção de relatórios.

Course: Scientific Writing Workshop, 5-7 July, 2004, Instructor: Dr. Elisabeth Heseltine, European Association of Scientific Editors

Course: Scientific Writing Workshop, 9-11 July, 2004, Instructor: Dr. Elisabeth Heseltine, European Association of Scientific Editors

European institute in statistical genetics. July 2004. Institute of Statistical Genetics of North Carolina State University in Raleigh (USA), University of Algarve, Portugal.

Seminário de Aquacultura e Novas Espécies. 12-15 Setembro 2004, Funchal, Portugal. Organização da Direcção Regional de Pescas (Governo Regional da Madeira, Secretaria Regional do Ambiente e Recursos Naturais), Com o apoio do CIMAR - Laboratório Associado. (Luís Conceição e Luisa Valente)

Workshop "Managing the Guadiana estuary – the Ecohydrology and Phytotechnology approaches "under the framework of the IHP-UNESCO, during 1-4 setember 2004 hosted by Luis Chícharo

Participation in scientific and advisory commitees

Chícharo, Luís. Participation as Scientific Expert in the EIFAC-SGRN meetings in Brussels, in June and December 2004. Support: European Commission (DGXIV)

Chícharo, Luís. Participation as Associated Expert in the Scientific Advisory Committee meeting on Ecohydrology, held in UNESCO, Paris, May 2004. Support: UNESCO

Chícharo, Luís. Participation as Chairman in the 3rd Coastal and Estuarine Ecohydrology WG meeting held in the University of Algarve (september 2004). Support: UNESCO